SUMMARY OF MAJOR RESEARCH PROJECTS AT THE EXPERIMENTAL LAKES AREA DURING 2004

6 December 2004

Research activity on site at the ELA during 2004 remained at a level comparable to 2003, well above the historic average. Approximately 180 different researchers, representing more than 20 different universities, government agencies and private companies, worked on site during the field season.

The experimental phase of the Cage Aquaculture study was in its second year, and this project was again the largest single project in terms of on-site activity. The METAALICUS study was in its fourth year of ecosystem-scale, experimental manipulation, albeit at a somewhat reduced level of on-site sampling activity. The estrogen (EDC) study moved into a monitoring and recovery phase, with no further estrogen additions to Lake 260 in 2004. With all field operations for the FLUDEX project completed in the fall of 2003, the work on this study focused on completion of site rehabilitation and publication of study results. The long-term, ecological research (LTER) program continued, but with very limited support from core funding. On-site meteorological monitoring moved into its 36th consecutive year. A group from the University of Alberta conducted several climate-change and acidification recovery experiments. Among other activities, several external groups conducted surveys of lake sediments and tree growth to assess long-term climatic history, and installed an automated, seismic monitoring station.

The following is an attempt to summarize the status of most major research projects by providing some information about their participants, purposes, designs and current status. These projects are grouped under several broad category headings.

Note:

Using information provided by research project leaders and other ELA staff, John Shearer compiled this summary. Where appropriate, the names of principal investigators, graduate students, and their affiliations are noted. However, DFO Experimental Lakes Area staff members and seasonal employees, many of whom have not been specifically mentioned, are conducting many aspects of most major projects. The summary is intended as an overview of research activities at the ELA during 2004. For more detailed information, the reader should contact those researchers responsible for each study, or refer to published literature.

TABLE OF CONTENTS

LONG-TERM MONITORING AND CLIMATIC FACTORS

In order to assess objectively the effects of anthropogenic perturbations on aquatic ecosystems, it is essential to systematically monitor non-perturbed systems over long time periods. Only thus can we hope to evaluate the effects of naturally-occurring events (weather, cyclic climatic oscillations) on these ecosystems and factor these effects into our interpretations of impacts resulting from human activities. Of course, natural perturbations also can have significant effects on processes within these small lake ecosystems.

Over more than three decades, researchers at the E.L.A. have been collecting data on natural lake ecosystems in support of, and as references for, the experimental studies. Increasingly, these data sets are becoming invaluable in their own right because of the unusual scope and length of the records.

LONG-TERM ECOLOGICAL RESEARCH (LTER) AND DATA MANAGEMENT

Principal Investigators:

- S. Kasian, LTER Coordinator and ELA Data Manager
- K. Beaty, P. Blanchfield, D. Findlay, D. Guss, L. Hendzel, R. Hesslein, M. Lyng, K. Mills, S. Page, M. Paterson, J. Shearer, M. Stainton, M. Turner.

All principal investigators are ELA/DFO staff.

Project Description and Goals:

In 1998 the Long-Term Ecological Research (LTER) project was established to co-ordinate the hydrological, chemical, and biological monitoring of long-term reference lakes at the ELA. Responsibilities for collection of meteorological data and management of the ELA multidisciplinary database were added to the project in 1999.

There are three objectives for the project:

1. To provide an envelope of expected natural variability against which experimental results can be assessed.
2. To provide a long-term record for the detection of change due to the effects of region-wide perturbances resulting from global stressors (e.g. climate change, atmospheric contaminant loading and stratospheric ozone depletion), for the assessment of variance and for the interpretation of ecological relationships.
3. To provide a secure and accessible database of ecological data collected at the ELA which serves the information needs of ELA researchers.

Activities in 2004:

Major changes in funding for Long Term Ecosystem Research and Data Management in 2004 resulted in a clear endangerment of the project. Primary funding (from the ELA operating budget) was reduced by 69% (from $73,500 to $23,000). The severely reduced budget was insufficient to cover the cost for water chemical analyses for lakes 239, 373, and 442, Lake 239 streams and precipitation; for summer student assistance for field sampling, hydrology and the chemistry lab; and for programming assistance for data management. Some long-term data sets could not be maintained.

Because ELA researchers considered this project to be so essential, they contributed funds and person days from a variety of other programs that directly benefit from the collected data. This one-time contingency funding allowed data collection, analyses and management to continue, for the most part, as in previous years.

Long-term records of meteorology and hydrology of the Lake 239 basin were maintained. Meteorological variables (air temperature, precipitation, wind speed and direction, bright sunshine and evaporation) were monitored daily. The 3 inflowing streams and outflow of Lake 239 were monitored for volume of flow (continuous record) and chemical composition (weekly). The five core lakes (114, 224, 239, 373, and 442) were again monitored, where possible, for all major disciplines which include: hydrology, water temperature, secchi depth and light extinction profiles, water chemistry, primary production, epilithon, phytoplankton populations and nutrient status, zooplankton, and fish. Discipline specific monitoring continued in other lakes to maintain long-term records.

Measurements of limnological variables and samples for chemistry, phytoplankton and zooplankton analyses were taken every 2 weeks through the open water season and twice over winter (shortly after ice-on and before ice-off). Surface water temperature was measured continually in all LTER lakes. Phytoplankton nutrient status (alkaline phosphatase activity and nutrient debt) was measured approximately every 2-4 weeks during the open water season in both epi- and meta-limnetic waters. Epilithon was synoptically sampled during midsummer. L239 was sampled twice for all components (metabolism, taxonomy and chemical composition) and L373 was sampled once for metabolism and twice for algal, bacterial and chemical composition and stoichiometry. Phytoplankton and zooplankton analyses included identification to species and biomass estimates. *Chaoborus* and *Mysis* in lakes 373 and 240 were sampled for comparisons to specific experimental lakes. Mark-recapture work to estimate fish populations occurred in spring and/or fall, depending on the species. Movements of lake trout and white suckers in Lake 373 were studied with acoustic telemetry for the purpose of comparison to those in the Aquaculture experimental lake. Cyprinid population data collection continued in the spring and fall in Lake 442 to provide specific reference information for the EDC experiment in Lake 260.

There were two new monitoring initiatives undertaken in 2004. All sampling stations will be exactly located with GPS and the information stored in the ELA Database. To date, lake centre buoys have been GPS-located. Monitoring of Lake 227, a long-term experimentally eutrophied lake, was added to the project

Progress continued on archiving data in the ELA Database, a secure and managed database, on improving the functionality of the Retrieval application and on developing day-to-day data management applications for researchers. Most core data sets were brought up to date with 2003 data. A new long-term data set of PAR (photosynthetically active radiation) was added to the ELA Database. All existing bathymetric maps for ELA lakes were scanned, brought up to date, and loaded into the Database. The Retriever was expanded so researchers can locate lakes on a regional map and obtain locations in various co-ordinate systems. More processed data options were added to the retriever to summarize chemical data in ways frequently requested. These include long-term plots and spreadsheets of raw data with percentiles, annual open-water epilimnetic means, and annual summaries of yields from monitored sub-basins in the Lake 239 drainage area. Improvements were made to the mass-balance modelling application. Database applications were built to manage continuously recorded data for fish tracking and lake surface temperatures. The littoral and zooplankton database applications were enhanced.

Summary of ELA Research for 2004

Major Findings or Conclusions:

Analyses of this year's data are still largely incomplete. See the list of presentations, publications, etc. for research pursuits.

In an audit of the Experimental Lakes Area, done by the Audit and Evaluation Directorate of DFO (September 2004), the Long-Term Ecological Research and Data Management project received a favourable review. The report states:

> "One of the most important contributions of research done at ELA is the long-term monitoring of natural ecosystems.", and
>
> "The facility's 30-year database helps research to be conducted more effectively."

The auditors recommended increasing the proportion of Environment Canada's funding towards operating the ELA meteorological site. Continued long-term meteorological records at ELA could be in jeopardy if a reasonable solution is not found.

Publications, Reports and Theses (with a significant LTER component):

a) Published

- Mills, K.H., E.C..Gyselman, S.M..Chalanchuk, and D.J..Allan. 2004. Growth, annual survival, age and length frequency distributions for unexploited lake whitefish populations. *Ann. Zoo. Fennici* **41**: 263-270.
- Hesslein, R.H., R.A. Dwilow, K.G. Beaty and M. E. Lyng. Chapter 10: A Comparison of Carbon Dioxide Net Production in Three Flooded Uplands (FLUDEX, 1999-2002) and a Flooded Wetland (ELARP, 1991-2002) Using a Dynamic Model. In Tremblay, A., Varfalvy, L. Roehm, C. and Garneau, M. (Eds.), 2005. *Greenhouse Gas Emissions: Fluxes and Processes, Hydroelectric Reservoirs and Natural Environments*. Environmental Science Series, Springer, Berlin, Heidelberg, New York, 732 pages.
- Hesslein, R.H. Chapter 23. Using Gas Exchange Estimates to Determine Net Production of CO_2 in Reservoirs and Lakes. In Tremblay, A., Varfalvy, L. Roehm, C. and Garneau, M. (Eds.), 2005. *Greenhouse Gas Emissions: Fluxes and Processes, Hydroelectric Reservoirs and Natural Environments*. Environmental Science Series, Springer, Berlin, Heidelberg, New York, 732 pages.

b) In Press:

- Findlay, D.L., M.J. Vanni, M. Paterson, K.H. Mills, S.E.M. Kasian, W.J. Findlay and A.Salki.2005. Dynamics of a boreal lake ecosystem during a long-term manipulation of top predators. Ecosystems (in press).
- Findlay, D.L., M. Paterson, H.J. Kling and Hendzel, L.L. 2005. Factors influencing *Gonyostomum semen* blooms in small boreal reservoirs. *Hydrobiologia* (in press)
- Mills, KH, Gyselman, EC, Chalanchuk, SM, and Allan, DJ. The population dynamics of unexploited lake whitefish (Coregonus clupeaformis) populations. Proceedings of 2002 great lakes lake whitefish-diporeia workshop. *Great Lakes Fishery Commission Tech. Rept.* (in press).
- Turner, M. A., D. B. Huebert, D.L. Findlay, L.L. Hendzel, R.A. Bodaly, and W.A. Jansen. 2005. Divergent impacts of experimental lake-level drawdown on planktonic and benthic plant communities in a boreal forest lake. Can. J. Fish. Aquat. Sci. (In revision).

- Weidman, P., M.A. Turner, and G.G. Goldsborough. 2005. The depth distribution of UV effects in the shallow littoral zone. J. North American Benthol. Soc. (In revision).

c) In Preparation:

- Baulch, H.M., D.W. Schindler, M.A. Turner, D.L. Findlay, P. Leavitt and M.P. Paterson. 2005. The effects of experimental warming on epilithic composition in a boreal lake. Limnol. Oceanogr.
- Baulch, H.M., M.A. Turner and D.W. Schindler. 2005. Effects of increased temperature on epilithic metabolism and potential implications of climatic change. *Limnol. Oceanogr.*
- Hesslein, R.H., M.A. Turner, D.Guss and M.Lyng. 2005. Distinguishing DOC-related changes in physical and chemical properties of a boreal lake due to climate variation and acidification. (In preparation)
- Turner, M.A., H.M. Baulch, S.M. Chalanchuk, I.J. Davies, D.L. Findlay, B.J. Hann, L. Hendzel, R.H. Hesslein, S.E.M. Kasian, D.K. McNicol, K.H. Mills, M.J. Paterson, C.G. Trick, and R.D. Vinebrooke. 2006. Biological disorder in a boreal forest lake recovering from acidification. *Ecosystems* (In preparation)
- Turner M.A., H.M. Baulch, S.E.M. Kasian, D.L. Findlay, R.D. Vinebrooke, and D.K. McNicol. 2005. Resilience and hysteresis in benthic algal associations of a boreal forest lake recovering from acidification. *J. North American Benthol. Soc*. (In preparation)
- Vinebrooke, R.D., M.A. Turner, D.L. Findlay, M. Paterson, and D.W. Schindler. 2005/6. Ecosystem functioning during anthropogenic stress and recovery: A 20-yr whole-lake experimental investigation. *Ecology Letters* (In preparation).

Long-term Data Sets Requested for Research Projects:

N.B. Long-term data sets extracted from the ELA Database by ELA DFO staff for their own research purposes are not tracked in detail. However, ELA staff made 104 queries of the database in the last 3 months (September-November 2004). The automatic logger of database activity was inoperative earlier in the year.

- Bristow, Corben. University of Ottawa. For modelling purposes, 2004 stream discharges for lakes 239 and 224 ouflow and 239 east sub-basin inflow, and 2004 meteorological data. These modelling efforts in part support the ELA aquaculture experiment.
- Vinebrooke, Rolf. University of Alberta. Phytoplankton data for Lakes 302S and 239 to examine species diversity and species extinction. This research supports the examination of biological recovery from acidification.
- Graham, Mark. University of Alberta. Lake 302S and 239 phytoplankton species data, air temperature, precipitation, and temperature profiles and heat budgets to examine biological recovery of acid-stressed boreal lakes in the context of a changing climate.
- Molot, Lewis. York University. Lake 239 and 227 phytoplankton and chemistry data to look at the correlation between Fe and prokaryotes. This research investigates the controlling factors determining the appearance of cyanobacteria blooms in fresh water lakes.
- Laird, Kathleen. Queens University. Lake 239 phytoplankton species, meteorological, hydrological and chemical data to ground truth observations from sediment cores. The research investigates the impacts of climate change on fresh water lake ecosystems.
- Curtis, Jeff. Okanogan University. Precipitation nutrient chemistry data.

- Schindler, David W. University of Alberta. Detailed record of chemical additions to Lake 227.
- Kelly, Carol. Rudd and Kelly Research Inc. Chemistry of Lake 239 inflow streams and precipitation for modeling nutrient inputs.
- Minns, Ken. DFO. Solar radiation data from the Met site for modeling trout habitat. The data will be used to develop models of availability of trout habitat in relation to climate change.
- Venkiteswaran, Jason. University of Waterloo. Water chemistry data from FLUDEX reservoirs for a poster presentation.
- Davis, Carla. Anishinaabeg of Kabapikotawangag Resource Council Inc. Taxonomic list of species to assist in a water quality study.
- Jones, Timothy. School of Biological Sciences, Bangor, Gwynedd,UK. DOC data from ELA lakes to help in a study of the effect of lake water DOC on the quality of drinking water.
- Beall, Fred. Great Lakes Forestry Centre. ELA DOC data for modelling the annual export of dissolved organic carbon in headwater streams across eastern Canada.
- DFO graduate students and post-docs (e.g. Brad Park, Julia Beveridge, Diane Orihel, Patricia Ramlal). A variety of physical limnology data for their theses and research.

Plans for 2005:

The future of the LTER and Data Management project is precarious due to funding pressures. Assistance in 2004 from other programs will not likely be available. However it is hoped that primary funding will be able to be somewhat increased in 2005. On the positive side, the shortfall is not large. The project will work with management to think of and pursue efforts to secure the necessary funds. Plans for 2005 will be dependent on the determined budget.

Specific Ancillary Studies:

Meteorological Monitoring

The ELA is the site of long-term monitoring of meteorological variables via a meteorological station (met site) that uses equipment provided by the Meteorological Service of Canada (MSC) of Environment Canada and is operated by ELA staff. Ken Beaty, with assistance from Mark Lyng, Ian Delorme, and others, has primary responsibility for this facility and data are contributed to the AES national climate database. In June of 2004, this site reached the milestone of 35 years of continuous monitoring record, making it an official long-term station in the AES Climate network. Meteorological variables (air temperature, precipitation, wind speed and direction, bright sunshine and evaporation) were monitored daily again in 2004.

These climatic data are essential for our understanding of interactions between climatic variables and the lake ecosystems we study.

<u>Canadian Air and Precipitation Monitoring Network (CAPMoN)</u>

ELA personnel, under the direction of K. Beaty, continued to operate a CAPMoN station at the ELA met site in 2004. The CAPMoN program, which monitors both atmospheric and precipitation chemistry at a network of sites across southern Canada, is funded and coordinated by the Meteorological Service of Canada. The ELA site monitors ground-level ozone, SO_2 and HNO_3 in the atmosphere, Cl, SO_4, NO_3, Na, NH_4, Ca, K, Mg, pH, and mercury in precipitation.

The ELA site, which has been operating since the 1980's, is frequently used as a baseline reference for sites in eastern Canada.

Canadian Network Isotopes in Precipitation (CNIP)

The ELA is a node in a Canadian network monitoring isotopes (^{18}O, Deuterium) in precipitation. This network (*http://sciborg.uwaterloo.ca/~twdedwar/cnip/ela.html*), coordinated from the University of Waterloo, comprises sites distributed broadly across Canada, including the high Arctic. Its current goal is "to discern fundamental linkages between the isotopic composition of precipitation and synoptic climate and to aid in designing and optimizing a more permanent future network". Ken Beaty is the ELA researcher responsible for the ELA site.

Phytoplankton Nutrient Status in ELA Lakes

Purpose or Goals:

Phytoplankton nutrient status measurements which include the use of composition ratios and physiological measurements (alkaline phosphatase, nitrogen debt, and nitrogen fixation activity) deal with the basic view that algae interacting with its environment provide direct and relevant answers regarding algal interactions within the aquatic food chain. Algal physiology and phytoplankton nutrient status explores the roles of essential nutrients (C, N, P) and physical factors in controlling algal species composition, succession and blooms, and chemical composition (lipids/carbohydrates, proteins, composition ratios, cell quotas) and determine to what extent laboratory studies can be applied to field situations. The species composition and biochemical composition of algae, together with other phytoplankton and zooplankton data can determine the efficiency of food chains, effect of perturbation, the production and consequences of harmful phycotoxins, and also the bio-availability of environmental toxic substances and their rates of removal from surface waters. As part of a continuing dataset, synoptic measurements of phytoplankton nutrient status were made on a selected number of lakes during 2004 in support of a number of ELA projects.

Principal Investigator:

- Len Hendzel (DFO, Freshwater Institute)

Work carried out during 2004:

This past summer was to have been the last year for nutrient status monitoring of the LTER lakes as well as Lake 302S (Acid Recovery), however work on these lakes was continued through 2004. Phytoplankton nutrient status (alkaline phosphatase activity and nitrogen debt) was measured on epilimnetic and metalimnetic water samples from LTER lakes 114 (epi only) 224, 239, 373, 442 and other ELA lakes (302S, 375 North and South) approximately every 2-4 weeks between early May and mid November.

Publications or Spin-off Research:

A major analysis of the ELA nutrient status data was planned for the summer of 2004, but was not initiated because of time limitations. A complete analysis of the data is planned for 2005. This will then be followed by the preparation of several manuscripts.

Plans for 2004:

Current plans call for no further nutrient status sampling of ELA lakes for 2005.

OXYGEN DYNAMICS IN LAKES, RIVERS AND GROUNDWATER: A NEW ISOTOPIC APPROACH

Principal Investigators:

- Jason Venkiteswaran, Ph.D. candidate, Dept. of Earth Sciences, University of Waterloo
- Dr. Sherry Schiff, Department of Earth Sciences, University of Waterloo, Waterloo, Ontario N2L 3G1

Objectives:

Continue to develop and deploy an oxygen stable isotope (^{16}O and ^{18}O) method of determining the balance of heterotrophy, autotrophy and atmospheric influence in lakes, rivers and groundwater in Canada.

Summary of Work carried out during 2004:

Two ELA lakes (L227 and L979) were each sampled hourly for 48 hours for O_2, CO_2, and DIC concentrations, and oxygen and carbon isotopic ratios at two locations in the lake. Both lakes were sampled at the lake surface and L227 was sampled at the O_2 maximum (approximately 0.5 to 1.0 m below the lake surface) and L979 was sampled at the bottom of the epilimnion (approximately 0.7 m below the lake surface).

Continuation of Study / Spin-off Research:

The diel data will provide a useful and interesting comparison between different methods of determining primary production, for example, between concentration and isotopic ratio techniques and between isotopic ratio techniques and ^{14}C incubations.

LONG-TERM CLIMATIC RECORDS IN THE WINNIPEG RIVER DRAINAGE BASIN

Three external research groups conducted sampling surveys within the Experimental Lakes Area during 2004 as part of a larger project to examine long-term climatic trends within the drainage basin of the Winnipeg River. The ELA sits astride a height of land which divides two major sub-basins of Winnipeg River drainage. This, coupled with the ELA existing long-term data sets, attracted these groups to the ELA for these surveys. The primary purpose of these studies is to assess the variability in climatic conditions in northwestern Ontario over the last 2 thousand years.

Algal Records in the Sediments:

Dr. Kate Laird and Dr. Brian Cumming of Queen's University cored sediments at selected locations in Lakes 239, 442, and 658 in July. These lakes were selected because of their size and depth, the simplicity of their basins, their headwater status, and the probability that their water levels responded significantly to previous periods of drought. The resulting cores will be analysed over the next few years for algal records that may denote periods of drought.

Sediment Sounding:

Dr. Mike Lewis and Dr. Thane Anderson from Natural Resources Canada (Bedford Institute) conducted acoustic profiling of lake sediments in Lakes 239 and 442 at the beginning of August. This work will complement the coring done by Laird and Cumming.

Tree Coring:

Scott St. George from the Geological Survey of Canada and the University of Arizona collected a number of cores from older trees in the ELA in mid July. He will be using these cores to link growth responses of these trees to periods of drought over the past two hundred years. This research is also part of Scott's Ph.D. studies at the University of Arizona. For more information, see http://www.ltrr.arizona.edu/~sstgeorg/Winnipeg_River_subsite/Winnipeg_River.htm.

SEISMIC MONITORING STATION

Natural Resources Canada, Seismology & Electromagnetism Section, installed an automated seismic monitoring station at the ELA in June of 2004. The station is located atop a bedrock ridge between Lake 239 and Roddy Lake, in the clearing created by the removal of the FLUDEX site 1 reservoir. Fully automated with a satellite data uplink, this is part of a small network of stations installed in northwestern Ontario and is expected to remain in place for 2 to 5 years.

HABITAT ALTERATION AND ECOSYSTEM PRODUCTIVITY

As humans have perturbed and manipulated aquatic ecosystems for various purposes, unexpected impacts have frequently occurred. Often these impacts have been manifested in major population shifts and alterations of energy flow within the food web. If we can better understand the factors which control system productivity and structure, and the food chain linkages affected by these perturbations, we will be better able to develop effective management and regulatory strategies for minimizing the adverse effects on aquatic ecosystems of many human perturbations. The following projects are intended to improve our knowledge of these linkages.

FERTILIZATION OF LAKE 227

Rationale:

Eutrophication remains one of the most common water quality problems in much of the world. As most ELA lakes are naturally oligotrophic, it has proved advantageous to maintain at least one study lake in which the primary productivity is elevated. This enables researchers to compare physical, chemical and food web characteristics in other ELA lakes with those in a more productive system, more typical of those in many areas of Canada, and elsewhere.

Research Activities:

Lake 227 was fertilized with phosphorus for the 36th consecutive year in 2004. This original ELA ecosystem-scale experiment was initiated in 1969 to demonstrate that atmospheric carbon dioxide could provide the carbon necessary for algal blooms in eutrophic lakes. Prior to 1990, all additions included various combinations of nitrogen and phosphorus. The ratio of phosphorus to nitrogen was changed during these previous stages of the experiment to test

whether this would influence the dominant algal groups. Since 1990, only phosphorus has been added. During 2004, phosphorus, as phosphoric acid, was again added to Lake 227 surface waters for twenty consecutive weeks (2.5 litres per week) during the ice-free season. The acid was diluted with lake water in a plastic barrel and dribbled via *Tygon* tubing into the near-shore water. The required acid was carried to the lake weekly. Sodium bicarbonate, to be used as a neutralizing agent in case of an acid spill, is stored on site.

We continued to monitor water chemistry, phytoplankton, and zooplankton in Lake 227 during 2004. Dr. Lewis Molot, of York University, has submitted a proposal to conduct enclosure studies of noxious cyanobacteria in this lake during 2005, utilizing the enhanced productivity of this system and the 36 years of historic data record during which the lake has been experimentally fertilized.

EFFECTS OF MACROPHYTE REMOVAL ON PIKE POPULATIONS

Principal Investigator:

Dr. Ken Mills, DFO Freshwater Institute

Rationale and Design:

Many property owners along lake shorelines remove rooted and floating plants (macrophytes) from the nearshore areas to facilitate water access for boating and swimming. However, these plants may provide important habitat for fish and other aquatic species. What are the impacts of mechanical removal of macrophytes from a small boreal lake?

Lake 191 at the Experimental Lakes Area was the site of a macrophyte removal experiment to determine the change in northern pike production when 50% of the macrophytes present in the littoral zone were removed. Changes in water chemistry and all trophic levels in the lake have been monitored during the study.

After two years of background study, macrophyte harvesting began in July 1996. A mechanical harvester removed fifty percent of the macrophytes present in the lake. The harvesting continued in 1997 and 1998. No macrophyte harvesting has occurred in the years following 1998.

We completed 5 years (1999–2003) of recovery monitoring of the macrophyte and fish community in the lake. As of 2003, the lake ecosystem appeared to be returning to a pre-experimental condition. However, no funding was available in 2004 to continue this monitoring, and there currently are no plans to resume monitoring in the future.

RECOVERY OF BOREAL LAKES FROM ACIDIFICATION

Despite substantial reductions in the release of acidifying emissions, large numbers of lakes in southeastern Canada and northeastern United States have not yet recovered from anthropogenic acidification. Also acid deposition continues to exceed critical loads across large portions of eastern Canada, causing acidification in these areas to continue, and threatening the biodiversity of these aquatic ecosystems. For example it has been estimated that even with full implementation of the Canada – USA Air Quality Agreement by 2010, about 76,000 lakes will remain chemically damaged (i.e., their pH will remain below 6 even though they could be expected to have pHs higher than 6). Similarly, Environment Canada's chemical models predict that about one quarter of the lakes in eastern Canada outside of the Sudbury area will still be

acidified after 2010. Moreover, integrated assessment models have predicted that about 160,000 Canadian fish populations will remain at risk.

The unexpected delays in recovery of many lakes appear to have been caused by several factors, including:

- Nitrogen oxide emissions have not been reduced as much as sulphur emissions;
- Some of the buffering components in precipitation have declined;
- Previously acidified watersheds are still releasing acidifying substances;
- Some of the buffering potential in the watersheds has been exhausted by prior acidification;
- Expectations for the recovery of aquatic ecosystems have often been incorrect and overly optimistic.

To better understand the natural recovery potential of boreal lakes, ELA researchers and colleagues have been studying several ecosystems that had previously been acidified experimentally. Beginning in 1974, ELA researchers had embarked on a major program of investigating the effects of acidification on lakes of the Boreal Shield. Several lakes (223, 114, and 302) and a wetland (239 Fen) were acidified using various experimental designs. This program provided scientific evidence that was instrumental in the development of the 1991 Canada-US Clean Air Act, which reduced sulphur emissions, partly in an effort to protect the integrity of acid-sensitive aquatic ecosystems.

RECOVERY OF LAKES 302 AND 223 FROM EXPERIMENTAL ACIDIFICATION - CONCLUSION

Goals and Purpose of the Study:

At the start of this research, comparatively little was known about the ability of aquatic ecosystems to recover from chronic acidification although much was known about the impacts of acidification. As a result, our studies of the natural potential of boreal forest lakes to recover from acidification have been particularly relevant.

The general goal of our acidification recovery studies has been to evaluate the ability of boreal forest lakes to recover from acidification without deliberate intervention in the recovery process to raise the pH of the lakes. To achieve this goal we have been studying the ability of two experimental lakes to recover from acidification in terms of their physical, chemical and biological properties as their target pH has been relaxed following years of experimental acidification. Although the principal experimental system was Lake 302S, we have conducted limited study of Lake 223.

The policy implications of our studies include indirect testing of the suitability of current acidifying emissions standards and, importantly, better defining the nature of recovery of boreal forest lakes from acidification. These goals support the Federal, Provincial and Territorial governments' commitment to the recent Canada-Wide Acid Rain Strategy for Post-2000. Our research is also proving to be an important component of the upcoming Canadian Acid Rain Assessment in 2004. The research also supports DFO's 1986 Policy for the Management of Fish Habitat by enhancing Canadians' ability to mitigate acid-related threats to the productive capacity of fish habitats.

Summary of ELA Research for 2004

Name(s) and Affiliation(s) of Major Participants/Contributors:

DFO members of the research group in 2004 (arranged alphabetically) were H. Baulch, K. Beaty, S. Chalanchuk, D. Findlay, R. Hesslein, L. Hendzel, S. Kasian, M. Lyng, K. Mills, S. Page, M. Paterson, and M. Turner (scientist-in-charge). EC partners included D. Jeffries, D. McNicol and R. Weeber. L. Armstrong (Ducks Unlimited) and C. Trick (U. Western Ontario) also participated this year. Colleagues from the U. of Alberta (R. Vinebrooke and his graduate students M. Graham, M. Christensen, and I. Phillips) have also been active participants.

Funding of the acidification research studies in 2004 was derived from several sources. Environment Canada (specifically D. McNicol of the Canadian Wildlife Service and D. Jeffries of the National Water Research Institute) provided operating funds that enabled us to continue long-term monitoring in Lake 302S and to conduct limited sampling in Lake 223. The Department of Fisheries and Oceans (DFO) provided salary support for its researchers, and provided support for the ELA platform, which was critical for these experiments. Funding of our U. of Alberta colleagues was obtained from DFO's Academic Subvention Grant, NSERC and the EJLB Foundation (described elsewhere in the 2004 ELA report).

Brief description of study activities in 2004:

During 2004, pH was unregulated in Lake 302S for the fourth year. We continued to monitor several physical (hydrology, temperature, and transparency), chemical (nutrient and ionic chemistry) and biological (phytoplankton, zooplankton and Chaoborus) properties of the pelagic zone. Our synoptic sampling in the littoral zone was restricted to benthic algae (epilithon). We also sampled the fish populations, and continued to monitor the success of the lake whitefish addition made to Lake 302S in 1999. We continued to sample Lake 223 to evaluate the recovery of lake trout, the permanence of changes in the white sucker population, and to find out whether *Mysis relicta* had recolonized the lake. With this study ending, several staff and partners have begun to focus upon analysing data, and to prepare reports and publications.

Several EC partners have been involved in various aspects of data analysis, including D. Jeffries, D. McNicol and R. Weeber. L. Armstrong (Ducks Unlimited) and C. Trick (U. Western Ontario) have also been contributing to data analysis and interpretation, as have colleagues from the U. of Alberta (R. Vinebrooke and M. Graham).

In addition, M. Graham and M. Christensen (U. of Alberta) conducted mesocosm studies in Lake 302S to examine the interactive effects of temperature and drought on pelagic foodweb structure. I. Phillips (U. of Alberta) performed an experimental re-introduction of the extirpated native crayfish Orconectes virilus in aquaculture cages. These studies are described in more detail at the end of this section.

New Publications:

ELA's acidification recovery research contributed significantly to the 2004 Canadian Acid Deposition Science Assessment, which is an Environment Canada publication (H. Morrison, ed.). Several ELA staff and colleagues were involved in the ELA contributions.

Only recently published articles and those in preparation are listed below. Primary scientific journal articles and theses related to this study (45 and 8, respectively, in the last decade) that have previously been listed in earlier annual reports are not repeated here.

- Graham, M.D., W. Keller, J. Heneberry, K.H. Nicholls, D.L. Findlay, M.A. Turner, D.W. Schindler, and R. Vinebrooke. 2005. Recovery responses of autotrophs to chemical improvements in atmospherically and experimentally stressed boreal lakes: the importance of scale. *Can. J. Fish. Aquat. Sci*. (In preparation).

- Hesslein, R.H., M.A. Turner, D. Guss and M. Lyng. 2005. Distinguishing DOC-related changes in physical and chemical properties of a boreal lake due to climate variation and acidification. (In preparation)
- Paterson, M., M.A. Turner, P. Chang, D.L. Findlay, W. Chang, D. Malley and L. Wesson. 2005/6. Changes in zooplankton community structure during and following a whole-lake acidification experiment. *Freshwater Biology* / *Limnol. Oceanogr*. (In preparation).
- Jeffries, D.R., M.A. Turner, R. Hesslein, S.E.M. Kasian, and R. Hecky. 2005. Hysteresis in the physicochemical properties of a boreal forest lake recovering from acidification. *Ecosystems* (In preparation).
- Jeffries, D.R., T. Clair, S. Couture, P.J. Dillon, C. Gagnon, S E.M. Kasian, W. Keller, D. McNicol, M.A. Turner and R. Weeber. 2005. Effects of climate on the coherence of the responses of boreal lakes in central and eastern Canada to reductions in sulphur deposition. (In preparation as of fall 2003)
- Turner, M.A., L.M. Armstrong, H.M. Baulch, D.L. Findlay, B.J. Hann, L. Hendzel, R.H. Hesslein, S.E.M. Kasian, D.K. McNicol, K.H. Mills, M.J. Paterson, C.G. Trick, and R.D. Vinebrooke. 2005. Biological disorder in a boreal forest lake recovering from acidification. *Ecosystems* (In preparation)
- Turner M.A., H.M. Baulch, S.E.M. Kasian, D.L. Findlay, R.D. Vinebrooke, and D.K. McNicol. 2005. Resilience and hysteresis in benthic algal associations of a boreal forest lake recovering from acidification. *J. North American Benthol. Soc*. (In preparation)
- Vinebrooke, R.D., M.A. Turner, D.L. Findlay, M. Paterson, and D.W. Schindler. 2005/6. Ecosystem functioning during anthropogenic stress and recovery: A 20-yr whole-lake experimental investigation. *Ecology Letters* (In preparation).

Current Plans for 2005 and Beyond:

2005 will be the first year that we are no longer intensively monitoring the recovery of Lake 302S; at most only very limited synoptic monitoring will be done of the lake ecosystem(s). Synoptic sampling of pelagic communities and water chemistry should occur during the stratified period if resources are available. At a minimum, we will maintain the integrity of curtains between the north and south basins of Lake 302. Otherwise team members and partners will continue to analyse data from the experimental whole-lake studies and from associated process-oriented research, and to publish the findings in scientific journals.

Although the experimental phase of this ELA research theme has ended, several research needs have been identified from our studies. Continued monitoring of the recovery of lakes 223 and 302S would help to distinguish whether the hysteresis observed during recovery represented lags in combination with stochastic events, or alternate endpoints for the study systems. Because littoral and pelagic responses often differed within equivalent functional groups, generalizations can not be made from one to the other (e.g., between phytoplankton and benthic algae or between zooplankton and zoobenthos).

Further quantification of delays in the recovery of biota is also needed to enable effective modeling of recovery. We also need a better understanding of the link between altered ecosystem structure and recovery of ecosystem function. Similarly, it remains to be confirmed that functional instability and structural variability were greater during pH recovery than during acidification. From a trophic perspective, because food web changes also shaped the character of recovery, the pattern of fluctuating abundances during initial reestablishment of taxa potentially intensifies ecosystem fluctuations and instability. However, it remains uncertain how persistent loss of biodiversity influences ecosystem function because Vinebrooke et al. concluded that several ecosystem processes could be unaffected by the loss of taxonomic richness.

Usually ecosystems are recovering from acidification in the context of multiple stressors. Hence, it is important to explore the consequences of how other forces might interfere with the recovery from acidification; including climate warming, exotic biotic introductions, eutrophication and contaminants. The establishment of parallel case studies varying in ecozone, degree (pH) and duration of acidification, and complexity of additional forces (e.g., climate, DOC, metals and nutrients) would be useful. In particular, further study is needed into the scale and forces affecting compression of habitat linked to lower DOC given that this phenomenon will be important for slow-growing cold-water stenotherms, which are also vulnerable to biotic invasions of warmer taxa related to climatic warming.

Ancillary Studies:

As part of the larger research focus on recovery from acidification and the potential multiple stressor linkages with climatic change, several smaller studies were conducted relative to Lake 302 South during 2004.

Interactive Effects of Temperature and Drought on Pelagic Food Web Structure

Investigators:

- Mark Graham, Ph.D. Candidate, Univ. of Alberta,
- Dr. Rolf Vinebrooke, Biological Sciences, Univ. of Alberta.

Activities in 2004:

Mark's Ph.D. research recently involved conducting a 2-factor mesocosm experiment in Lake 302S this past summer, which examined the interactive effects of temperature and drought on pelagic foodweb structure. His two-factor design involved 9 treatment combinations, which were each replicated three times for a total of twenty-seven 2000-L mesocosms. To date, bacterial counts have been completed, and currently, phytoplankton enumerations are taking place.

His preliminary findings will be presented at the Annual Meeting of the American Society of Limnologists and Oceanographers in Spain (June 2005). Mr. Graham receives financial support from NSERC (PGS-D scholarship), and the research is supported by NSERC, DFO Academic Subvention, and EJLB Foundation funds awarded to Dr. Vinebrooke.

Quantitative Analyses of the Phytoplankton Community of Lake 302S

Investigators:

- Mark Graham, PhD Candidate, Univ. of Alberta
- Dr. Rolf Vinebrooke, Biological Sciences, Univ. of Alberta.

Activities in 2004:

Mark is currently completing quantitative analyses of taxonomic changes in the phytoplankton community of Lake 302S from 1980 to 2003. He is placing these findings in a larger geographic context by comparing them with changes in 22 acid-stressed lakes in central Ontario over a similar 20-yr period.

Some of these findings were presented by Mr. Graham in a poster at the annual meeting of the Ecological Society of America in Portland (August 2004). Also, this manuscript will be ready for submission to CJFAS by January 2005.

Warming x Drought x Acidification Mesocosm Experiment

Investigators:

- Michael Christensen, M.Sc. Candidate, Univ. of Alberta
- Dr. Rolf Vinebrooke, Biological Sciences, Univ. of Alberta.

Activities in 2004:

Mr Christensen conducted a 3-factor (warming x drought x acidification) mesocosm experiment in Lake 302S this past summer to examine the impacts of multiple stressors on pelagic food web structure. His experiment design was replicated three times for a total of twenty-four 2000-L mesocosms that were sampled at 10-day intervals over a 50-day period. Michael has completed all bacterial enumerations, and is currently starting phytoplankton counting.

His preliminary findings will be presented at the Annual Meeting of the American Society of Limnologists and Oceanographers in Spain (June 2005). Mr. Christensen receives financial support from NSERC (PGS-D scholarship), and the research is supported by NSERC, DFO Academic Subvention, and EJLB Foundation funds awarded to Dr. Vinebrooke.

Experimental Re-introduction of the Extirpated Native Crayfish Orconectes virilus

Investigators:

- Iain Phillips, M.Sc. candidate, Univ. of Alberta
- Dr. Rolf Vinebrooke, Biological Sciences, Univ. of Alberta

Activities in 2004:

Mr. Phillips performed an experimental re-introduction of the extirpated native crayfish, *Orconectes virilus*, using 10 aquaculture cages (4 m^2 area), which were deployed in late May along a 1-m depth contour along the southern shoreline of Lake 302S. Iain monitored benthic invertebrate and periphyton communities on a bi-weekly basis until mid-August. Key responses variables that are currently being analyzed include the total biomass, taxonomic composition, and stable-isotopically inferred trophic structure and carbon sources of zoobenthos and periphyton.

Iain will be presenting his preliminary findings at either the meeting of the North American Benthological Society in New Orleans (May 2005) or ASLO 2005 in Spain (June 2005).

RESERVOIR IMPACTS

In Canada, reservoirs are generally created primarily for generation of hydroelectricity. Many cause flooding over large areas of northern wetland and forest land. The water levels in these reservoirs tend to be drawn down during the winter periods when electrical demand is high and water flows are low.

Over the past decade, ELA researchers have been investigating the ecological effects of flooding caused by reservoir creation and operation. In most cases, this has involved experimental alteration of water levels, as a simulation of what typically occurs during the creation and operation of reservoirs. The focus of these studies has been the production and fate of methylmercury and various greenhouse gases.

This work is now winding down, but data analyses are ongoing and a study of possible mercury mitigation using low concentrations of selenium is planned for 2005.

EXPERIMENTAL LAKES AREA RESERVOIR PROJECT (ELARP)

Objective:

The Experimental Lakes Area Reservoir Project (ELARP) is a whole-ecosystem flooding experiment designed to examine the production and mobilization of methylmercury (MeHg) in response to flooding, and to determine if reservoirs are significant sources of the GHG's carbon dioxide (CO_2) and methane (CH_4) to the atmosphere.

Design:

In June, 1993, following two years of background studies, the outflow of a ELA Lake 979 and its surrounding wetland was dammed, and the water level raised 1.4 meters to flood 14 hectares of peatland. Direct by-products of the decomposition of the flooded vegetation in the peatland are CO_2 and CH_4. Mobilization of MeHg within the flooded ecosystem and release to the atmosphere of CO_2 and CH_4 in response to the flooding were monitored intensively. A non-flooded wetland system (ELA Lake 632), was monitored as a reference. Following winter drawdown, flooding of Wetland 979 was repeated in summer and fall of 1994 and 1995, as detailed studies continued in both wetland systems.

During the open-water periods of 1996 through 1998, the 979 wetland was experimentally flooded, but the system was studied less intensively. GHG emissions and MeHg mass-balance budgets were monitored. In 1999 and again in 2000, the system was flooded, but no ecosystem monitoring was conducted. During the open water period of 2001, the system was flooded once again and a regular monitoring program was carried out.

Flooding was repeated in 2002, 2003, and 2004, but only minimal general monitoring was conducted. However, researchers from the University of Waterloo continued one special study, as described below.

Fluxes and Isotopic Composition of Greenhouse Gases in a Flooded Wetland - Ten Years Post Flood.

Project Goal:

To determine if the flooding and drawdown of a boreal wetland over ten years has changed the production and flux of greenhouse gases using concentration data, stable carbon isotopes (^{12}C and ^{13}C) and vegetation coverage.

Principal Investigators:

- Jason Venkiteswaran, Ph.D. candidate, Department of Earth Science, University of Waterloo

- Dr. Sherry Schiff, Department of Earth Science, University of Waterloo, Waterloo, ON, N2L 3G1

Work completed in 2004:

In summer 2004, L979 centre buoy, L979 east inflow, and L240 outflow samples were collected for DIC, CH_4, and O_2 concentration, as well as stable carbon and oxygen isotopes. To determine the diffusive flux rate and isotopic ratio of the CO_2 and CH_4 from the wetland surface to the atmosphere, static flux chambers were deployed at ten sites on the wetland. To determine the ebullitive (bubble) flux and isotopic ratio of the CO_2 and CH_4 from the wetland surface to the atmosphere, vertical profiles of bubbles entrapped within the flooded peat were sampled.

Continuation of Study / Spin-off Research

This research extends the static flux chamber data set and, for the first time, elucidates the role of ebullition in the total flux of greenhouse gases from the wetland surface. The bubble sample techniques developed to sample the L979 wetland will be employed at other sampling sites. The ebullition data may be the last segment of the greenhouse gas cycle within the wetland that had not been well studied before.

FLOODED UPLAND DYNAMICS EXPERIMENT (FLUDEX)

Purpose and Approach:

The purpose of the Upland Flooding Experiment (FLUDEX; Flooded Upland Dynamics Experiment) was to study the greenhouse gas and mercury impacts of flooding forested upland areas. Three forested uplands, a moist forest and two dry forested areas, located in the watershed of Roddy Lake were flooded in the summers of 1999 to 2003, inclusive, to create experimental hydroelectric reservoirs. Greenhouse gases fluxes before and after flooding were measured at all three sites. Carbon dioxide, methane and nitrous oxide were monitored. Fluxes are being compared to the previously flooded boreal wetland (ELARP project) and to existing hydroelectric reservoirs to determine the potential greenhouse gas contribution of global freshwater reservoirs. The production of methyl mercury from flooded soils and the bioaccumulation of methyl mercury through the food chain were measured in the experimental reservoirs. Mitigation strategies that will have direct planning application are being investigated. The fifth year of flooding (2003) was the last for the experimental reservoirs. Studies on the effectiveness of selenium additions to reduce mercury in food chains began in 2003 with a survey of mercury and selenium levels in reservoirs, wetlands and natural lakes at the ELA and in northern Quebec.

Principal Investigators:

- Project coordination:
    - Drew Bodaly (Freshwater Institute)
- Hydrology and project design:
    - Ken Beaty and Mark Lyng (Freshwater Institute)
- Mercury dynamics:
    - Kristofer Rolfhus, James Hurley and David Krabbenhoft (University of Wisconsin and USGS)
    - Britt Hall and Vincent St.Louis (University of Alberta)

- Katharine Peech and Michael Paterson (University of Manitoba and Freshwater Institute)
- Drew Bodaly and Andrew Majewski (Freshwater Institute)
- David Findlay (Freshwater Institute)
- Mariah Mailman (University of Manitoba)
- Greenhouse gases and carbon decomposition:
- Elizabeth Joyce, Cory Matthews and Vincent St.Louis (University of Alberta)
- Natalie Boudreau, Jason Venkiteswaran and Sherry Schiff (University of Waterloo)
- Len Hendzel (Freshwater Institute)

Study Schedule and Plan:

1998-99: Dam construction, background data collection including site characterization of vegetation, mercury inventories in soils and vegetation, carbon inventories, and set up of pump and piping water supply system hydrological network completed.

1999-00: Year 1 of flooding, measurement of greenhouse gas (GHG) emissions and mercury dynamics.

2000-01: Year 2 of flooding, study of GHG emissions and mercury dynamics.

2001-02: Year 3 of flooding, study of GHG emissions and mercury dynamics; beginning of studies on the use of controlled burns to mitigate mercury problems in boreal reservoirs.

2002-03: Year 4 of flooding, study of GHG emissions and mercury dynamics; completion of studies on controlled burning.

2003-04: Year 5 (last year) of flooding, study of GHG emissions and mercury dynamics; removal of reservoir dikes and rehabilitation of sites; initiation of studies on the possible use of selenium additions to reservoirs to mitigate mercury problems.

The three areas flooded were: a High Carbon moist forest (Site 1), a Medium Carbon dry forest (Site 2), and a Low Carbon very dry forest with areas of exposed bedrock (Site 3). The amount of organic carbon stored on the sites before flooding was found to be highest in Site 1, intermediate in Site 2, and lowest in Site 3. The approximate sizes of each of these impoundments were as follows:

Site	Area (ha)	Mean depth (m)	Volume (10^4 m^3)	Dike length (m)
Site 1	0.69	1.0	0.69	190
Site 2	0.50	0.8	0.40	130
Site 3	0.66	1.2	0.79	350

Reservoirs were filled with water pumped from Roddy Lake using a diesel-powered unit. Water from all sites drained back to Roddy Lake. Water renewal times of approximately 10 days were maintained during the open water season. Maximum dike height and reservoir depths were approximately 2 m.

Site Rehabilitation:

Upon completion of final sampling in October 2003, all water pipes were removed. Removal of the reservoir dikes and site rehabilitation was completed in 2003 and early 2004. The rehabilitation was conducted according to guidelines prescribed by the Ontario Ministry of Natural resources and included bulldozing and on-site burning of the dead jack pine trees in the

former reservoirs. The diesel pump, fuel tank, and storage trailer were also removed as part of this rehabilitation process.

Funding Support:

The FLUDEX project was funded from a variety of sources, including Manitoba Hydro, Hydro-Québec, the Department of Fisheries and Oceans, the Natural Sciences and Engineering Research Council (Canada), the United States Geological Survey, and the Centre for Research in Earth and Space Technology (Ontario).

Primary Publications:

- Bodaly, R.A., K.G. Beaty, L.L. Hendzel, A.R. Majewski, M.J. Paterson, K.R. Rolfhus, A.F. Penn, V.L. St.Louis, B.D. Hall, C.J.D. Matthews, K.A. Cherewyk, M. Mailman, J. P. Hurley, S.L. Schiff and J.J. Venkiteswaran. 2004. Experimenting with hydroelectric reservoirs. *Environmental Science and Technology* **38**: 347A-352A.
- Hall, B.D., V.L. St. Louis, and R.A. Bodaly. 2004. The stimulation of methylmercury production by decomposition of flooded birch leaves and jack pine needles. *Biogeochemistry* **68**:107-109.
- Hall, B.D, V.L. St.Louis, K.R. Rolfhus, R.A. Bodaly, K.G. Beaty and M.J. Paterson. In press. The impact of reservoir creation on the biogeochemical cycling of methyl and total mercury in boreal upland forests. *Ecosystems*.
- Hendzel, L.L., C.J.D. Matthews, J.J. Venkiteswaran, V.L. St. Louis, D. Burton, E. M. Joyce and R.A. Bodaly. Accepted for publication. Nitrous oxide (N_2O) fluxes in three experimental boreal forest reservoirs. *Environmental Science and Technology*.
- C.J.D. Matthews, E.M. Joyce, V.L. St.Louis, S.L. Schiff, R.A. Bodaly, J.J. Venkiteswaran, K.G. Beaty, and B.D. Hall. In press. Carbon dioxide (CO_2) and methane (CH_4) production in small reservoirs flooding upland boreal forest. *Ecosystems*.
- Mailman, M., and R.A. Bodaly. Total mercury, methyl mercury, and carbon in fresh and burnt boreal plants and soil. Accepted for publication in *Environmental Pollution*.

Conference Presentations:

- Bodaly, R.A., K.G. Beaty, R.J.P. Fudge and D. Huebert. 1999. Introduction to the upland flooding experiment. Air and Waste Management Association Conference on Mercury in the Environment, Minneapolis.
- Hall, B.D., V.L. St.Louis, and R.A. Bodaly. 1999. Impact of reservoir creation on the biogeochemical cycling of methylmercury in boreal forest uplands. Air and Waste Management Association Conference on Mercury in the Environment, Minneapolis.
- Rolfhus, K.R., J. Hurley, and D.P. Krabbenhoft. 1999. The burden and mobilization of total and methyl mercury from upland soils at the Experimental Lakes Area, Ontario, Canada. Air and Waste Management Association Conference on Mercury in the Environment, Minneapolis.
- Hall, B.D., V.L. St.Louis and R.A. Bodaly. 1999. Production of methylmercury in three experimental boreal upland reservoirs. Canadian Society of Limnologists, Annual Meeting, Edmonton.

- Joyce, E. and V.L. St.Louis. 1999. Concentrations of carbon dioxide and methane in three experimental boreal upland reservoirs. Canadian Society of Limnologists, Annual Meeting, Edmonton.
- Hall, B.D., V.L. St.Louis, R.A. Bodaly and K.G. Beaty. 2000. Methylmercury production in flooded forest uplands. Society of Environmental Toxicology and Chemistry, Nashville.
- Rolfhus, K.R., J.P. Hurley, and K.P. Krabbenhoft. 2000. The dynamics of mercury species fluxes from inundated upland boreal forest soils. Society of Environmental Toxicology and Chemistry, Nashville.
- Bodaly,R.A., R.J.P. Fudge, M.J. Paterson, K.A. Peech, and L. Wesson. 2000. Methyl mercury bioaccumulation in the food chains of three experimental reservoirs that flooded boreal forest uplands. Society of Environmental Toxicology and Chemistry, Nashville.
- Matthews, C.J.D. V.L. St.Louis, and R.A. Bodaly. 2001. The impact of reservoir creation on greenhouse gas fluxes from three forested upland subcatchments. International Society of Limnology, Melbourne, Australia.
- Paterson, M.J., C. Podemski, R.A. Bodaly, and K.A. Peech. 2001. Bioaccumulation of methyl mercury by invertebrates from four reservoirs and three natural lakes at the Experimental Lakes Area, Ontario, Canada. Sixth International Conference on Mercury as a Global Pollutant, Minamata, Japan.
- Rolfhus, K.R., J.P. Hurley, B.D. Hall, and D.P. Krabbenhoft. 2001. The response of soil/water mercury fluxes to periodic inundation of upland boreal forest reservoirs. Sixth International Conference on Mercury as a Global Pollutant, Minamata, Japan.
- Hall, B.D., V.L. St.Louis, R.A. Bodaly, and K.G. Beaty. 2001. Impacts of reservoir creation on the biogeochemical cycling of methylmercury in boreal forest uplands. Sixth International Conference on Mercury as a Global Pollutant, Minamata, Japan.
- Bodaly, R.A., A.R. Majewski, W.A. Jansen, R.J.P. Fudge, and M.J. Paterson. 2001. Uptake of mercury by fish in three experimental reservoirs that flooded forested boreal uplands. Sixth International Conference on Mercury as a Global Pollutant, Minamata, Japan.
- Hall, B.D., V.L. St.Louis and R.A. Bodaly. 2001. Methylmercury production in flooded birch leaves and jack pine needles. Sixth International Conference on Mercury as a Global Pollutant, Minamata, Japan.
- Peech, K.A., M.J. Paterson, R.A. Bodaly and L. Wesson. 2001. Methylmercury n zooplankton in reservoirs that flooded boreal forest catchments: The Flooded Uplands Dynamics Experiment. Sixth International Conference on Mercury as a Global Pollutant, Minamata, Japan.
- Hall, B.D., Bodaly, R.A., and 8 co-authors. 2002. Mercury cycling and greenhouse gas fluxes from flooded boreal forest uplands: the FLUDEX (FLooded Uplands Dynamics EXperiment) Project at the Experimental Lakes Area. American Society of Limnology and Oceanography, Victoria. (invited)
- Mailman, M. and R.A. Bodaly. 2004. Is Selenium depressed in recently flooded hydroelectric reservoirs? Seventh International Conference on Mercury as a Global Pollutant. Ljubljana, Slovenia.
- Mailman, M. and R.A. Bodaly. 2004. Mercury and methyl mercury in fresh and burnt soils and vegetation. Seventh International Conference on Mercury as a Global Pollutant. Ljubljana, Slovenia.

- Mailman, M. and R.A. Bodaly. 2004. The burning question: does burning before flooding lower methyl mercury and greenhouse gases. Seventh International Conference on Mercury as a Global Pollutant. Ljubljana, Slovenia.
- Majewski, A.R., R.A. Bodaly et al. 2004. Mercury in the aquatic food chains of three experimental boreal upland reservoirs. Seventh International Conference on Mercury as a Global Pollutant. Ljubljana, Slovenia.
- Jansen, W.A., R.A. Bodaly et al. 2004. Time course of elevated Hg concentrations in fish in hydroelectric reservoirs of northern Manitoba, Canada. Seventh International Conference on Mercury as a Global Pollutant. Ljubljana, Slovenia.
- Bodaly, R.A. and 7 co-authors. 2004. The power of whole lake ecosystem experimentation in solving problems of environmental contamination. COMERN Annual Conference, Gimli, Manitoba.
- Rolfhus, K.R., B.D. Hall, R.A. Bodaly, J.P. Hurley. 2004. Methylmercury production in experimental reservoirs inundating boreal forests: Sensitivity to land cover and flooding. California Bay-Delta Authority Science Conference 2004, Sacramento, California.

Theses Completed:

- Boudreau, Natalie M. 2000. Soil carbon, carbon dioxide, and methane in three experimentally flooded upland boreal forest reservoirs: a 13C inventory of sources and processes. *M.Sc. thesis*, University of Waterloo, Department of Earth Sciences, 180 p.
- Hall, B.D. 2003. Impacts of reservoir creation on the biogeochemical cycling of methylmercury in boreal forest uplands. *Ph.D. thesis*. Department of Biological Sciences, University of Alberta, 231 p.
- Joyce, E. M. 2001. The impact of experimental reservoir creation on greenhouse gas fluxes from forested uplands. *M.Sc. thesis*, Department of Biological Sciences, University of Alberta, 70 p.
- Matthews, C.J.D. 2002. Greenhouse gas production in experimental reservoirs flooding upland boreal forest. *M.Sc. thesis*, Department of Biological Sciences, University of Alberta, 101 p.
- Peech Cherewyk, K.A. 2002. Methylmercury bioaccumulation in zooplankton: an assessment of exposure routes and accumulation in newly flooded reservoirs. *M.Sc. thesis*, Department of Entomology, University of Manitoba, 89 p.
- Mailman, M. 2003. Effect of burning before flooding on mercury and greenhouse gases. *M.Sc. thesis*, Department of Zoology, University of Manitoba, 173 p.

MERCURY LOADING AND BIOACCUMULATION

Certain substances, when released into natural ecosystems, may persist for years in a toxic form, and may bioaccumulate within the food chain to create health problems for higher organisms, including humans, particularly when exposures are chronic.

While such persistent toxicants are often experimentally studied under laboratory conditions, only studies conducted in real ecosystems can effectively examine the complexity of ecosystemic pathways and compartments in which these substances move and accumulate.

Some controlled experimentation in real ecosystems is required to validate existing and proposed regulatory standards for these substances.

Current studies at the ELA, both on a whole watershed scale, and in various mesocosms, are helping to answer the questions about mercury contamination in aquatic biota, particularly fish, and delineate the linkages between mercury in fish and the mercury that is deposited from the atmosphere.

MERCURY EXPERIMENT TO ASSESS ATMOSPHERIC LOADING IN CANADA AND THE UNITED STATES (METAALICUS)

Background and Rationale:

The presence of methylmercury (MeHg) in fish is a natural occurrence, but fish MeHg concentrations in remote lakes in eastern Canada, including Ontario, are often above the 0.5 ppm limit for commercial sale. This is the case even in the absence of direct anthropogenic discharges of mercury to the lakes. Methylmercury is produced from inorganic mercury by bacteria, and is accumulated preferentially by fish. Fish with elevated mercury concentrations are a health concern due to the toxicity of MeHg and exposure via fish consumption. There is a general consensus that elevated MeHg concentrations of fish in remote lakes are influenced by inputs of atmospheric inorganic mercury directly to lakes and indirectly via their watersheds. Furthermore, anthropogenic mercury emissions are likely contributing to mercury loading rates to lakes. Anthropogenic inputs originate as emissions from coal combustion, waste incineration, and as emissions from other industrial and mining processes.

Much research has been undertaken to better understand the impacts of mercury emissions on fish mercury concentrations, and the potential effectiveness of emissions controls. In North America alone, emission controls for electric utilities have been estimated to cost billions of dollars per year. Despite this research activity, a fundamental question remains unanswered: What will happen to fish mercury concentrations if atmospheric mercury deposition is reduced? Uncertainty remains regarding both the magnitude and timing of the response.

The METAALICUS experiment (Mercury Experiment To Assess Atmospheric Loading in Canada and the US) is currently underway at the Experimental Lakes Area (ELA) to examine this issue. METAALICUS involves the addition of stable, non-radioactive, mercury isotopes to a whole ecosystem to see if there is a response in mercury concentrations fish. Pilot scale studies began in 1999 and the full scale experiment began at Lake 658 in 2001. Mercury has been added to the Lake 658 ecosystem each year since 2001, and permission has been obtained to continue the loading phase through 2006.

Experimental Objectives:

METAALICUS is designed with the following overall objectives:

- To determine the relationship between the atmospheric deposition of mercury to a lake ecosystem and the MeHg concentration of fish.
- To determine the response time of MeHg in a whole ecosystem, including fish, to changes in rate of atmospheric deposition of mercury (Hg(II)).
- To establish the relative importance of mercury deposited on uplands, wetlands, or onto the lake surface as sources of MeHg to fish.

Participants:

Principle Investigators:

R.A. Bodaly, DFO Winnipeg, and R. Harris, Tetra Tech Inc., Toronto

Other DFO Winnipeg Investigators:

M. Paterson, P. Blanchfield, C. Podemski, K. Sandilands

Other Investigators:

M. Amyot, Université de Montréal, Montréal, QC
C. Babiarz, U. of Wisconsin, Madison, WI
B. Branfireun, U. of Toronto, Toronto, ON
C. Gilmour, Smithsonian Institute, MD
H. Hintelmann, Trent University, Peterborough, ON
J. Hurley, U. of Wisconsin, Madison WI
C. Kelly, R & K Research Inc., Saltspring Is., BC
D. Krabbenhoft, USGS, Madison WI
S. Lindberg, Oakridge National Laboratory, Oakridge, TN
J. Rudd, R & K Research Inc., Saltspring Is., BC
V. St.Louis, U. of Alberta, Edmonton, AB
M. Tate, USGS, Madison WI

International Advisory Panel:

J. Munthe, Swedish Environmental Research Institute (IVL).
E. Swain, Minnesota Pollution Control Agency
R. Hesslein, Department of Fisheries and Oceans, Winnipeg.
J. Wiener, University of Wisconsin, Lacrosse

Study Description:

As described above, METAALICUS is a whole-ecosystem experiment in which mercury loading to a headwater lake and its watershed is being altered experimentally. Lake 658 at ELA was selected for the study. It is a small (8.4 ha), low productivity, headwater lake on the Canadian Shield and is one of the lakes reserved for research at the ELA. Background studies documenting site conditions prior to the experiment were carried out in 1999-2000 and are discussed in the 2001 *Summary of Major Research Projects at the ELA*.

Mercury is being added in the form of stable, non-radioactive isotopes of inorganic mercury (Hg(II)). The power of using isotopes lies in the ability to follow the newly deposited mercury separately from background mercury. Furthermore, different mercury stable isotopes are being added to the upland, wetland and lake surface (^{200}Hg, ^{198}Hg and ^{202}Hg respectively) to determine the relative contributions of these sources to fish mercury levels.

ELA is a low deposition area for mercury, with approximately 7 µg/m^2/yr of wet mercury deposition. The low mercury deposition rate at ELA means that adding the equivalent of about 1/6th of a teaspoon (approximately 12.5 g) of mercury per year increases wet Hg deposition to the 52 ha Lake 658 ecosystem (lake and watershed) by 3-5 fold. This addition results in a mercury wet deposition rate to the experimental system that is comparable to rates currently observed in some parts of the US Northeast and Florida.

Mercury concentrations are being tracked in all major compartments in the lake, watershed, and atmosphere. Detailed process studies are also being carried out to follow the movement and transformations of mercury through the watershed and lake, as well as air/surface exchange of

mercury. This process-based approach will allow us not only to document what happens, but also to understand why. This is essential if we are to use the results of the study to make predictions for other locations. The approach is providing critical information for an existing model that predicts fish mercury concentrations in lakes and the effects of remedial actions such as reductions in mercury loading.

The experiment is being carried out in two phases. Phase I involved pilot and baseline studies in 1999-2000, to prepare for Phase II. The ELA Management Board approved Phase I studies at the February 1999 and February 2000 meetings. Final approval of the full-scale experiment for 2001 through 2003 was obtained in March 2001. Permission to continue adding mercury to the ecosystem for the 2004-2006 periods was granted in February 2004.

Milestones:

- (1999-2000) Two years of pilot scale experiments; pre-addition background monitoring of both candidate lakes.
- (2001-2004) Four years of whole-ecosystem isotope additions to upland and wetland areas of the watershed and to the lake surface, and continued pilot studies.
- (2005 and 2006) The loading phase of the experiment will be continued if funding is obtained.
- (2007 and beyond) Monitoring of the food web methylmercury concentrations until conditions return to pre-addition levels.

Public Consultation:

During 2000, public information meetings were conducted in Dryden and Kenora to discuss the project with the public. In addition a presentation was made to three NGO's at a meeting in Toronto. Feedback from these presentations was positive. It should be noted that there has been no public opposition or negative media coverage in connection with METAALICUS since permission was originally granted and the project began. Scientific, public, and governmental feedback has been very positive.

Pilot Scales Studies:

Pilot-scale studies from 1999-2003 were described in the *Summaries of Major Research Projects at the ELA* for 2000, 2001, 2002 and 2003, based on results available at the time. These included:

- Isotopic Hg(II) additions to small upland plots;
- Isotopic Hg(II) additions to a wetland plot; and
- Additions of isotopic mercury to lake enclosures

In 2004, some of the pilot-scale studies done from 1999-2003 were continued or expanded to gain additional knowledge.

Additions of Isotopic Mercury to Lake Enclosures

During 2000-2001, a study of isotopic additions of ^{200}Hg(II) to four enclosures was carried out at Lake 239 at the ELA. The enclosure experiments in 2000-2001 confirmed for the first time that the new isotopic analytical methods being used could indeed follow added mercury isotopes through the water, sediments and food web, including fish.

In 2002 and 2003, 11 corrals were set up in ELA Lake 240, and dosed with varying amounts of mercury, up to 15X current annual wet deposition at ELA. In 2003, a different isotope was used than in 2002.

Whole-Ecosystem Mercury Additions at Lake 658

Lake 658 Research Activities:

After two years of pilot and baseline studies in 1999-2000, METAALICUS went “full scale” in June 2001. Stable non-radioactive Hg(II) isotopes are being applied to the upland, wetland, and directly to the lake surface in the Lake 658 watershed at ELA. The upland, wetland and lake are receiving ^{200}Hg, ^{198}Hg, and ^{202}Hg respectively. In each case the application rate is approximately 20-25 µg m^{-2} yr^{-1} in terms of the total amount of mercury being added. Isotopes have been applied during the 2001-2004 field seasons. Upland and wetland areas are being sprayed once per year by an airplane flying low over the canopy. Isotopes are being applied directly to the lake by mixing from a boat at a depth of 0.7 m, over a series of 9 applications during the ice-free season. The mercury loading rate directly to the lake surface is approximately 22 µg m^{-2} yr^{-1}.

Impact on Downstream Lakes:

Based on pilot-scale studies and our knowledge of the behaviour of mercury in ELA lakes, most of the added mercury will be bound to particles (soils, peat, sediments) in the Lake 658 ecosystem or returned to the atmosphere in the long term. Mercury in the Lake 658 outflow enters a very large downstream lake (Winnange Lake). We expect that the added mercury isotope will not be detected in the Winnange Lake food chain due to the small amount that will be discharged from Lake 658 and the very large volume of Winnange Lake relative to Lake 658 (approximately 1000x larger). Monitoring is being carried out in Winnange Lake to verify that the Winnange Lake food web is not impacted by the experiment. The concentrations of the three different mercury isotopes added to the Lake 658 watershed were examined in young-of-the-year yellow perch and northern pike prior to the addition of isotopes (June 2001) and two years after mercury additions began (May 2003). None of the mercury isotopes added as part of the METAALICUS study is presently detectable in Winnange Lake fish or sediments. This is not surprising considering that the transport of mercury isotopes out of Lake 658 has been measured and determined to be very small.

2005 Field Season:

The 2005 field season will be the fifth field season during which whole ecosystem mercury additions are planned. Detailed monitoring of site conditions, mercury concentrations, and the fate and transport of mercury will be undertaken. Pilot-scale studies will also continue.

Lake Restoration:

Prior to the beginning of METAALICUS, it was anticipated that MeHg concentrations in the food web following the mercury additions would be within the range presently observed in remote Canadian lakes that do not receive local anthropogenic mercury sources. This has been the case to date. If fish mercury concentrations do increase significantly in Lake 658, as a result of METAALICUS, it is expected that concentrations will return to background levels after mercury additions are stopped.

After the experiment has been completed, the study lake will be monitored until fish mercury concentrations return to pre-addition levels and the lake returns to conditions specified in Section VII. 3. of the *ELA Memorandum of Agreement*. During this recovery period, concentrations of mercury in fish and sediments in Winnange lake will also be monitored every second year.

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ENDOCRINE DISRUPTING CHEMICALS (EDCS)

Humans are producing and releasing to the environment a number of chemicals which are structurally similar to naturally occurring endocrine substances or hormones. There is considerable evidence that some of these manufactured chemicals, intentionally or otherwise, imitate natural hormones and, under certain conditions, disrupt normal endocrine functioning in a number of species. Can these chemicals, when present in lakes and streams, disrupt the endocrine functioning, including reproduction, of fishes? If so, what are the potential consequences?

EFFECTS OF A POTENT ESTROGEN MIMIC ON AQUATIC POPULATIONS - A WHOLE-LAKE ADDITION EXPERIMENT

Project Team:

Freshwater Institute:

[1]Karen Kidd, Principal Investigator
Paul Blanchfield
Bob Evans
Dave Findlay
Ken Mills
Vince Palace
Michael Paterson
Cheryl Podemski
Alex Salki

[1]Current address: Biology Department, University of New Brunswick, Saint John, NB

Other Agencies:

Scott Brown, Environment Canada, Burlington, Ontario
David Graham, Department of Civil and Environmental Engineering, University of Kansas
Karsten Liber, Toxicology Centre, University of Saskatchewan
Mark McMaster, Environment Canada, Burlington, Ontario
Glen Van Der Kraak, Department of Biological Sciences, University of Guelph
Greg Toth, Mark Bagley, and Jim Lazorchak, US EPA, Cincinnati

Background:

Considerable evidence exists that aquatic organisms are being exposed to and impacted by a wide range of compounds that mimic hormones. Fish exposed to these compounds often exhibit an array of responses including depressed circulating sex steroid levels, reduced gonad size and fecundity, and males have become feminized through the development of egg proteins and eggs. One of the most sensitive and common tools used to assess exposure to endocrine disrupting chemicals (EDCs) is the presence of vitellogenin (VTG), an egg yolk protein precursor, in the plasma of male fish. Recent studies have shown elevated plasma VTG in male fish downstream of sewage treatment plants (Harries et al. 1997; Jobling et al. 2002).

Natural and synthetic estrogens such as estriol and 17α-ethynylestradiol (EE2), two of the main active components of birth control pills, are present at ng/L concentrations in sewage effluents (Kolpin et al. 2002; Kirk et al. 2002; Tilton et al. 2002). Though other estrogenic compounds are present in these effluents, the natural and synthetic estrogens are believed to be posing the greatest threat to the endocrine systems of the resident fish populations. Laboratory studies have confirmed that these compounds are causing the feminization and elevated VTG levels observed in male fish and developmental problems in females downstream of sewage plants (Kramer et al. 1998; Lange et al. 2001; Miles-Richardson et al. 1998; Parrott et al. in press; Schultz et al. 2003; Jobling et al. 1996).

Despite the overt physiological evidence that fish are being adversely impacted by EDCs, it remains unclear whether these compounds are impacting a population's sustainability. It has been recognized nationally and internationally that there is a need to determine whether the molecular and cellular effects, such as VTG production, observed in fish exposed to EDCs are indicative of changes in population viability (Campbell and Hutchinson 1998; Arcand-Hoy and Benson 1998). Though significant progress has been made in characterizing the effects of hormone mimics on individuals, population-level approaches to identify and quantify effects are lacking.

Purpose of Experiment:

This whole ecosystem study was developed to determine whether aquatic populations are being adversely impacted by EDCs, and to determine the relationship between organism- and population-level responses to these compounds. This six-year study (1999-2004) will determine the impacts of the synthetic estrogen, EE2, on well-defined fish and invertebrate populations at the Experimental Lakes Area (ELA). EE2 was chosen for this experiment because it is a potent estrogen mimic that is known to affect the endocrine system of fish and other vertebrates. EE2 acts directly and effectively upon the endocrine system of organisms, and, therefore, research results are broadly applicable to field and laboratory studies of other estrogen-like compounds.

The main objective of this study is to determine the ecological relevance of molecular, cellular and organism-level screening tools currently used to assess the exposure of freshwater organisms to EDCs. In addition, this experiment will determine 1) the magnitude, mechanisms, and timeframe of EDC impacts on fish populations, 2) the impacts of an EDC on lower-trophic-

level organisms, and 3) the most sensitive species and life history stages of freshwater biota exposed to an EDC. Results will be critical in determining whether EDCs are impacting the viability of freshwater populations, in interpreting the ecological relevance of assessment data from studies by DFO and other Departments (e.g. Environment Canada studies on fish downstream of sewage treatment plants), in identifying sentinel species for field studies, and in developing the science used by regulators and industries for ecological risk assessments, mitigation strategies and release regulations for EDCs.

Study Site and Experimental Design:

Lake 260 was chosen for this whole-lake addition experiment; it has a surface area of 34 hectares and a maximum depth of 14.4 m. This lake has been part of a long term monitoring program at the ELA and considerable data exist on its limnological, physical, and biological characteristics. It supports well-defined lake trout (*Salvelinus namaycush*) and white sucker (*Catostomus commersoni*) populations of approximately 300 and 500 individuals, respectively, as well as the small fish species fathead minnow (*Pimphales promelas*; used extensively in laboratory EDC assays) and pearl dace (*Semotilus margarita*). Nearby Lakes 442, 224, 114, Roddy (468) and 373 are being used as reference systems throughout this study because most are similar in physical and chemical characteristics, have the same fish species and long-term data on their populations, and some historical data on lower-trophic-level biota.

Two years of baseline research (1999 & 2000) and three years of EE2 additions (2001-2003) have now been completed. In 2004, we did not add EE2 to the lake in order to monitor continued impacts and/or recovery of the lake and its biota. The following is a summary of what has been accomplished to date in 2004 by the various collaborators on this experiment.

EE2 Additions and Water Column Concentrations

In the summers of 2001-2003, EE2 was added three times weekly to the surface waters of Lake 260 to maintain low, but environmentally-relevant, concentrations of this potent estrogen in the surface waters. Methods of additions and quantification have been described in previous reports to the ELA Management Board. The mean concentrations of EE2 in the epilimnion of the Lake were 6.0 ± 2.9, 5.1 ± 1.8, and 4.8 ± 1.0 ng/L in 2001-2003, respectively. Monthly surface water samples were collected from May until October in 2004 to determine whether there are residual levels of EE2 in the water. These samples are still being analysed and these data will be available in the winter of this year.

Effects of EE2 on Fish

Fish Population Studies:

We sampled the fish populations in Lakes 260, 442 and 224 during spring and fall of 2004 in a similar fashion to previous years. Sampling was done in a manner that minimizes mortality, and the data are being used to assess age and size distributions, sex ratios, age to maturity, condition factors, abundance, growth rates, and annual survival and recruitment for lake trout and white sucker. These data and those collected from the reference systems during this study will be used to assess annual variability in unmanipulated populations, and to determine the effects of EE2 on fish populations in Lake 260. Lake trout were caught and sampled using trap nets and short sets of gill nets in the fall while white sucker were caught and sampled in trap nets in the spring. Other species - fathead minnow, pearl dace, lake chub, and slimy sculpin - were caught in trap nets and sampled in spring and fall in each lake. Most of the spring and fall

data from 2004 are still being analysed. Lake trout abundances for Lake 260 have been calculated for 2003 (latest data). White sucker data are still being compiled and analyzed.

Population sizes of fathead minnow and pearl dace were evaluated using a mark-and-recapture program on Lakes 260 and 442 in 1999-2004. The data are based on a fall marking period of 10 days and a spring recapture period of 10 days. Each time, 30 baited minnow traps are used daily. In the fall, fish are released during the marking period, whereas in the spring, the fish are caged over the recapture period.

Because effects in longer-lived populations will take time to manifest, we hope to continue netting in 2005-07 to determine the impacts of an estrogen mimic on fish sustainability and to assess the ecological relevance of biomarkers used to determine estrogen exposure in fish. At present, there are no funds to continue with this research; it is our hope to find support prior to the 2005 field season in order to adequately assess the population-level impacts of EE2.

Fathead Minnow Reproductive Behaviour:

Laboratory studies have shown that estrogen exposure affects the reproductive behaviour of fathead minnows (Majewski et al. 2002). It is not known whether such impacts would occur in fish under natural conditions. Underwater video was used to record spawning behaviour of male fathead minnows in Lake 260 and in reference lakes.

Fish Fertilization Success:

Laboratory studies have shown decreased survival and skewed sex ratios of fish larvae exposed to estrogen mimics (Parrott et al. in press). In this study, fertilizations of lake trout eggs have been done during 5 successive fall spawning seasons in the study and reference lakes to evaluate % fertilization, survival, growth and hatching success, and for developmental abnormalities. Fry survival and development is being assessed in the laboratory at the Freshwater Institute. Lake trout were captured using trap nets in Lakes 260 and 442 and in Roddy Lake during fall 1999-2003. Eggs were collected from 10 females in Lake 260 and 5 females in Lake 442 during 1999. In 2000, 5 females in Lake 260, 5 in Lake 442 and 1 from Roddy Lake were spawned. In 2001, 5 females from Lake 260, 5 from Roddy Lake (Lake 468) and 6 from Lake 442 were spawned. In 2002, 5 females from Lake 260 and 6 females from Lake 442 were spawned. In 2003, 6 females from Lake 260 and 7 females from lake 442 were spawned. Individual egg diameters and total egg volumes were recorded to calculate fecundities for each female. Unfertilized eggs were transported in sterile plastic bags back to the Freshwater Institute in Winnipeg where they were fertilized with a composite of milt obtained from male (>3 males) lake trout from the same lake. Eggs were reared at 8^{o}C with subsamples collected and preserved for measures of gross embryonic development and thyroid hormone and vitamin analysis.

Molecular and Cellular Responses in Fish:

The purpose of this component of the project is to assess the biochemical and tissue-level effects of EE2 exposure using both baseline data collected from fish populations in Lake 260 and three years of reference lake data. Several parameters are being examined and include vitellogenin (VTG; egg protein precursor) and its mRNA production, sex steroids, thyroid hormones, and gonadal development.

To avoid confounding interpretations of population-level effects, a small percentage (less than the annual natural mortality) of the lake trout, white sucker, fathead minnow and pearl dace populations were sacrificed to obtain gonad weights, fecundities, and gonad sections for histology and steroidogenesis. Fish were sampled from Lakes 260, 442, and Roddy Lake (Lake

468) (and Lake 114 for minnows) in 2004 in conjunction with the spring and fall netting programs for the population-level research.

Vitellogenin:

Plasma samples were collected each fall from male and female lake trout and white sucker to assess the production of the egg protein precursor vitellogenin (VTG).

Fathead minnows and pearl dace were collected for VTG analyses in the spring and fall of 1999-2004.

Circulating Sex Steroids and Steroid Production:

Alterations in circulating levels of the major biologically active reproductive steroid hormones are a common response in fish following exposure to endocrine disrupting substances. Reductions in steroid levels have been correlated to reductions in gonadal development, reduced expression of secondary sexual characteristics, increased age to maturation and altered fecundity in fish populations (McMaster et al., 1991). Detailed mechanistic studies examining the pituitary-gonadal axis, identified reduced gonadal steroid productive capacity as one of the major contributors to these reduced circulating steroid levels (Van Der Kraak et al., 1992; McMaster et al., 1996).

As part of this study, we collected plasma from white sucker and lake trout from three lakes, prior to EE2 additions, in the fall of 2001-2003 following exposure, and in 2004 for recovery. We measured the two biologically active steroids in both sexes, testosterone and 17ß-estradiol in females and testosterone and 11-ketotestosterone in males, and compared levels between sites as well as to the other reproductive endpoints being measured.

In the fall of 2002, 2003 and 2004, we also conducted *in vitro* gonadal incubations on female white sucker ovarian tissue from all three of the lakes to determine the steroid biosynthetic capacity of the ovarian tissue and to determine whether EE2 addition altered this reproductive endpoint. Incubations were conducted under both basal incubation conditions as well as following stimulation with human chorionic gonadotropin (hCG).

In the small forage fish species (pearl dace) we used the *in vitro* gonadal incubation procedure to compare the ability of gonadal tissue to produce steroid hormones following EE2 additions. This procedure has been used previously in fish too small to obtain sufficient amounts of blood.

Impacts on Gonad, Kidney and Liver Tissues:

Tissue sections from fathead minnow were examined in fish collected from the study and reference lakes in 1999-2004. Liver cells in the EE2-exposed individuals were enlarged when compared to baseline data from Lake 260 and reference lake fish in the fall of 2001. Hepatocyte volume index (HVI) is determined by counting the number of nuclei within a specific microscopic field of view and is a measure of the relative size of liver cells (Leatherland and Sonstegard 1984).

Gonadal development in fish was assessed using histological sections to determine whether EE2 exposure affects the timing and maturation of testes and ovaries in white sucker, pearl dace and fathead minnow. Medial sections of ovaries were collected and were examined for state of maturation, presence of atretic follicles, frequency distribution of oocyte stages, lesions and the presence of intersex (development of both male and female tissues). Testes were examined for delayed testicular maturation, inhibited spermatogenesis, asynchronous cyst maturation, seminiferous lobule deformities, replacement of generative tissue with connective tissue and other lesions (intersex/testis/ova).

The oocyte size and differential counts for pearl dace have not yet been completed for the 2004 samples.

Effects of EE2 on Lower Trophic-level Biota

Effects on Phytoplankton and Bacteria:

Bi-weekly water samples were collected from these lakes in conjunction with zooplankton samples for phytoplankton and chemical analyses (phosphorous and nitrogen, chlorophyll *a*, suspended carbon and nitrogen). Bacteria samples were also taken from Lake 260 and reference Lakes 373 and 239 over the same time period.

Impacts of EE2 on Zooplankton:

Vertical water column tows were collected bi-weekly from ice-off to ice-on in 2004 as was done in 1999-2003 on Lake 260 and on the reference lakes. All zooplankton samples from 1999-2002 and most from 2003 have been identified and counted to determine abundances, community composition, and sex and egg ratios (used to estimate birth and mortality rates

Other Lower Trophic Level Biota:

Monthly samples of the zooplankton predator *Chaoborus* spp. have been taken through 2004. Samples collected in 2004 are currently being counted (Mike Paterson, Freshwater Institute).

In previous years we examined the effects of EE2 on leeches (1999-2003), and larval and adult aquatic insects (1999-2001) using traps and artificial substrates. No research was done in 2004 on these organisms.

We have also conducted studies on the effects of EE2 on mink and green frog larvae and tadpoles. No research was done on these organisms in 2004. A publication on this research is currently in review (see below).

Training:

Two graduate students have been trained as part of this experiment. B. Park was a M.Sc. student at the University of Manitoba that was looking at the effects of EE2 on resident tadpoles. He completed his thesis in August of 2003. J. Werner is a Ph.D. student at the University of Manitoba that is conducting studies on the fertilization success and biochemical responses of fish in Lake 260 exposed to EE2.

Future Research Schedule:

The collaborators on this project will be meeting in March of 2005 to review results from the past field seasons. At present there are no funds to continue research on this experiment. We are seeking funds to continue with the fish population monitoring (at minimum) and some basic plankton monitoring for another 2-3 years. Continued research on the lake will allow us to determine whether the fathead minnow and pearl dace populations are recovering from EE2 exposure or whether there are ongoing impacts on these species. In addition, we would continue to look for any delayed effects of EE2 on the longer-lived white sucker and lake trout

Summary:

- Continuous EE2 additions have been completed for three summer seasons. Mean concentrations of EE2 in Lake 260 were 6.0, 5.1 and 4.8 ng/L over the 5 months of additions in 2001-03, respectively.

- The surface waters of Lake 260 were sampled monthly in 2004 to look for residual concentrations of EE2. These samples are still being processed.

Conclusion:

By exposing well-defined aquatic populations to a known and potent EDC, we are determining whether estrogen mimics affect the reproductive success of organisms under wild conditions where EE2 is the only stressor. This information is critical for determining the ecological relevance of the screening tools currently used to assess effects of EDCs on aquatic biota in both laboratory and field studies. Linking organism-level responses to impacts on populations will also improve our ability to assess the risks that EDCs pose to wildlife.

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Manuscripts Published, In Review or In Preparation:

- Werner, J., Palace, V.P., Kidd, K.A. Reproduction success in wild lake trout and the effects of ethynylestradiol. In preparation.
- Blanchfield, P., Kidd, K.A., Palace, V.P., Mills, K., Lazorchak, J. and Lattier, D. Population-level effects of a potent estrogen: results from a whole lake experiment. In preparation.
- Podemski, C., Kidd, K.A., K. Dsyzy and M. Dobrin. Effects of a potent estrogen on leeches. In preparation.
- Palace, V.P., Wautier, K.G., Evans, R.E, Blanchfield, P., Mills, K., Chalanchuk, S., Godard, D., McMaster, M., Tetrault, G., Peters, L.E., Vandenbyllardt, L. and K.A. Kidd. Biochemical and histopathological effects of ethynylestradiol in pearl dace (*Semotilus margarita*) exposed to the synthetic estrogen in a whole lake experiment. Environ. Toxicol. Chem. In review.
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Recent Conference Presentations:

- Lazorchak, J., Palace, V., Evans, R., Wautier, K., Flick, R., Wiechman, B., Braam, A, Kidd, K., Lattier, D., Molecular, Protein and Histological Indicators in Fathead Minnow and Pearl Dace Exposed to EE2 in a Whole Lake Dosing Experiment . SETAC 25th Annual Meeting, Portland, Oregon, 14-18 November 2004.
- Kidd, K., Palace, V., Blanchfield, P., Mills, K., Wautier, K., Lazorchak, J., Lattier, D. Differences in the biochemical- through population-level responses of two minnow species exposed to a potent estrogen mimic. SETAC 25th Annual Meeting, Portland, Oregon, 14-18 November 2004.
- Kidd K., Paterson, M., Salki, A., Findlay, D., Blanchfield, P, Mills, K. Responses of a freshwater food web to whole-lake additions of a potent estrogen. SETAC 25th Annual Meeting, Portland, Oregon, 14-18 November 2004.
- Kidd, K.A., Paterson, M., Salki, A., Mills, K., Blanchfield, K. Effects of a potent estrogen mimic on aquatic populations. Aquatic Toxicity Workshop 25-27 October 2004.
- Palace, V., K. Kidd, P. Blanchfield, K. Mills, R.E. Evans, C.L. Baron, and K. Wautier. Vitellogenin induction and histopathological effects in pearl dace (*Semotilus margarita*) captured from a lake experimentally treated with the synthetic estrogen ethynylestradiol. SETAC 24th Annual Meeting, Austin, TX, 10-13 November 2003.
- Lazorchak, J.M., Flick, R., Lattier, D., Toth, G., Kidd, K., Palace, V., Evans, B., Mills, K., and T. Hodge. Vitellogenin gene expression in fathead minnows exposed to EE2 in a whole lake dosing experiment. SETAC 24th Annual Meeting, Austin, TX 10-13 November 2003.
- Park, B. and K. Kidd. Effects of 17α-ethynylestradiol on development and gonad differentiation of larval ranids: a lake exposure. SETAC 24th Annual Meeting, Austin, TX 10-13 November 2003.
- Kidd, K., C. Podemski, A. Salki, M. Paterson, D. Findlay, K. Liber, J. Lazorchak and C. Watson. Impacts of whole-lake synthetic estrogen additions on lower-trophic-level biota. SETAC 24th Annual Meeting, Austin, TX 10-13 November 2003.

ENVIRONMENTAL IMPACTS OF AQUACULTURE

As wild fish populations come under increased pressures from human exploitation, commercial aquaculture or “fish farming” has become increasingly important as a source of fish protein for humans. While most Canadian aquaculture has focused on marine systems, there is increasing interest in freshwater aquaculture, in the Great Lakes and potentially in smaller inland systems. Until now, little research has focused on the environmental impacts of such activities in freshwater lakes.

IMPACTS OF CAGE AQUACULTURE ON LAKE ECOSYSTEMS

Goal or Purpose of the Study:

This whole ecosystem study has been developed to assess the environmental and ecological impacts of cage aquaculture under current industry practices. The three-year study will determine the impacts of aquaculture on water quality, primary production, sediments and native invertebrate and fish communities. A mass balance approach and the measurement of stable sulphur, carbon and nitrogen isotopes will be used to trace the movement of aquaculture-related waste materials in the ecosystem.

Major Participants/Contributors:

Participants:

- Cheryl L. Podemski, Freshwater Institute, Fisheries & Oceans
- Ken Mills, Freshwater Institute, Fisheries & Oceans
- Michael Paterson, Freshwater Institute, Fisheries & Oceans
- Michael Turner, Freshwater Institute, Fisheries & Oceans
- Paul Blanchfield, Freshwater Institute, Fisheries & Oceans
- Ray Hesslein, Freshwater Institute, Fisheries & Oceans
- Lori Tate, Freshwater Institute, Fisheries & Oceans
- Len Hendzel, Freshwater Institute, Fisheries & Oceans
- Dave Findaly, Freshwater Institute, Fisheries & Oceans
- Paula Azevedo, Freshwater Institute, Fisheries & Oceans
- Karen Kidd, University of New Brunswick at St John
- Marilynn Kullman, MSc candidate, University of New Brunswick at St John
- Corben Bristow, MSc candidate, University of Ottawa
- Rebekah Rooney, MSc candidate, University of Manitoba
- Sherry Schiff, University of Waterloo
- Dominic Bureau, University of Guelph

Contributors:

- Aquaculture Co-operative Research and Development Program
- Northern Ontario Aquaculture Association
- Meeker Aquaculture
- Aquacage Fisheries
- Martin Mills
- NSERC

2004 Study Activities and Preliminary Results:

Farm Operation:

On May 31, 2004, a team of over 30 ELA staff and students moved approximately10,250 female rainbow trout into the net pen in Lake 375. There were 156 mortalities in the 2 weeks following fish introduction. Total mortality over the summer was approximately 5%, the same as in 2003. Fish were harvested on November 1, 2004. Fish remained healthy throughout and no antibiotic use was required. Total feed usage over the production cycle was 9671 kg. Estimated total production was 9759 kg

Limnology:

On a monthly basis, samples for water quality analysis were collected along depth profiles in both the north and south basin of the lake. Also on a monthly basis, but on alternate weeks (resulting in biweekly sampling), depth-integrated samples were collected from the epi, meta, and hypolimnion at the same stations. During spring and fall turn-over, additional depth integrated samples were collected and analyzed for all forms of phosphorus and nitrogen. Sediment traps were collected weekly in both basins to determine sedimentation rates of carbon, nitrogen, and phosphorus. Many of the water chemistry samples remain to be analyzed. On a biweekly basis, a YSI multi-parameter probe was used to construct meter by meter depth profiles for temperature, oxygen, pH, conductivity, turbidity, and fluorescence at 12 stations placed along the north-south axis of the lake. Secchi depths were also determined at each station.

This project offered an opportunity to study the impacts of aquaculture waste production (i.e. waste food, metabolism products) on nitrification-denitrification at the sediment surface. It was hypothesized that N_2O emissions would increase if there were any substantial accumulation of uneaten food or waste products of fish metabolism accumulating on the sediments in the area beneath the fish pen. In order to determine this, water column N_2O concentrations at two stations in lake 375 (375 N & S) and in one control lake (373) have been monitored before and during the past two seasons (2003-04) of operation of an experimental fish enclosure.

Phytoplankton and Bacteria:

Phytoplankton and bacteria were sampled bi-weekly from the deep stations in the north and south basins throughout the ice-free season in 2004. Samples have been analysed and data are in the process of being worked up. The phytoplankton community in both basins of Lake 375 prior to the aquaculture cage addition were typical for oligotrophic shield lakes

Algal physiology and phytoplankton nutrient status measurements, which include the use of composition ratios and physiological measurements (alkaline phosphatase, nitrogen debt, and nitrogen fixation activity), explore the roles of essential nutrients (C, N, P) and physical factors in controlling algal species composition, succession and blooms, and chemical composition (lipids/carbohydrates, proteins, composition ratios, cell quotas). The species composition and biochemical composition of algae, together with other phytoplankton and zooplankton data, can determine the efficiency of food chains, effects of perturbation, the production and consequences of harmful phycotoxins, and also the bio-availability of environmentally toxic substances and their rates of removal from surface waters. Phytoplankton nutrient status (alkaline phosphatase activity and nitrogen debt) was measured on epilimnetic and metalimnetic water samples from lakes 375 N&S, and L373, as well as a number of other ELA lakes, approximately every 2-4 weeks between early May and mid November

Bacterial biomass and turn-over rates in Lake 375 for 2004 have been analysed.

Littoral Periphyton:

Our benthic algal sampling program was designed to evaluate the hypothesis that if phosphorus (and nitrogen) is mobilized from the aquaculture operation, we can expect the productive capacity of the littoral zone to increase. From a metabolic perspective we would expect to observe increased photosynthetic potential because of the high dissolved inorganic carbon in L375, which alleviates carbon limitation typical of epilithic biofilms in ELA lakes. From a food-quality perspective, we also expect that if the availability of phosphorus (and nitrogen) are increased, stoichiometry (especially C:P) of littoral biofilms will be enhanced. Note though that timelines associated with nutrient dynamics will likely vary with the nutrient and in terms of sensitivity to detection by a mid-summer synoptic sampling program. Although N might become available during the open-water season if ammonia from fish wastes is soluble and exceeds pelagic demand, P release from the fish waste will likely depend upon cycling through the anoxic hypolimnion and require entrainment into the epilimnion during spring and fall overturns.

In 2004, L375 and L373 were sampled synoptically once each for metabolic information and twice each for taxonomy + chemical composition.

Periphyton Trays:

In order to simulate littoral shorelines in closer proximity to cage operations, six Plexiglas trays holding 8 ceramic tiles (2x2 cm^2) were placed in Lake 375 and 1 tray placed in reference Lake 373 in May 2004. In addition, 1 tray was placed directly inside the cage. The trays were positioned in a transect 50, 100 and 150 m north and south of the fish cage at a depth of 1 m. The trays were sampled monthly with 2 tiles per tray being removed. The September sample represented accumulated seasonal growth of periphyton. In addition 2 sets of tiles were placed directly on bedrock, at a depth of 0.5 m, allowed to colonize, and sampled it August. These were used as a comparison to natural community structure. All samples have been analysed.

Zooplankton:

In 2004, we continued to collect samples to estimate the abundance, biomass, and species composition of zooplankton and invertebrate predators (primarily *Mysis relicta*) in Lake 375 and reference Lake 373. These organisms are important food for fish and also act as indicators of changes in water quality. Samples were collected at multiple stations on a biweekly basis for zooplankton and at monthly intervals for *Mysis*. To date, we have completed counting of samples collected from 2001 to 2003. Data for 2004 are not yet available.

Sediments and Associated Biota:

To examine the impacts of the farm on productivity of lake benthos, on a biweekly basis sediment cores were collected by a KB gravity corer along depth transects (11, 13, 15, 17, 19, 21m) in both basins of L375 and in the reference lake (L373). Samples were collected at 11m and deeper because experience has shown that hard (rock) substrates in shallower waters of these two lakes prevent operation of the corer. Samples were sieved through a 250µm sieve and were preserved in 10% formalin. These samples are being archived until funds for their analyses are obtained.

In order to examine the near-field impacts of the farm on sediments and benthic invertebrates, between May and September 2004, sediment core samples were collected along a distance transect from the aquaculture cage in L375. The transect ran along the 15m isobath and had sites located directly beneath the cage, at the cage edge, and 1m, 2m, 5m, 15m, and 45m from the cage edge. From July to September, additional sampling sites were added at 10m and 20m

The top 2 cm of sediment was extruded from three replicate cores from each distance. Pore-water was collected via filtration and ammonia concentration was measured using an ion selective electrode.

Four replicates were collected to be subsampled for sediment nutrient and metals analysis; these samples are currently being prepared for analysis. From May to June 5 replicates were washed with a 250µm mesh net for invertebrate analyses; after July this number of replicates was increased to seven. In all samples, species composition will be determined where possible and individual biomass will be determined using a digitizing system and published length-weight relationships. This analysis is yet to be completed.

Sediment Bioassay:

The spatial extent and magnitude of the impacts of aquacultural waste on the benthic community were assessed in 2004 through a sediment bioassay of the fingernail clam *Sphaerium simile*. Survival, growth, reproduction, and metal body burdens were evaluated for individual clams exposed for 6 weeks to sediment collected from directly below, and from 1, 3, 5, 8, and 50m in a transect away from the edge of the fish cage. This sediment bioassay is an effective method for testing the impacts of cage culture on the growth, reproduction and survival of benthic invertebrates such as the fingernail clam.

Energy Transfer to the Native Food Web:

Little is known about the effects of cage culture on the native food web. The main objective of this component of the study is to assess whether the aquaculture fish feed or trout faeces are being used as a novel energy source by the Lake 375 biota. To achieve this, we are examining the carbon, nitrogen, and sulphur stable isotope signatures of invertebrates and fish collected before and after the introduction of cage aquaculture into L375. Adding to the 2002 and 2003 collections, aquatic invertebrates, macrophytes and periphyton were collected monthly from May to August 2004 from L375 and the upstream reference lake, L373. For each collection date, all invertebrates were sorted into families and frozen. Zooplankton and *Mysis* were collected monthly from May through October. Samples of fish were collected in the spring and fall of 2004, and minnows were collected regularly throughout the summer. Samples will be processed in winter 2004/2005. Samples of water, sediment, and fish faeces are being analyzed by Dr. S. Schiff's lab at the Earth Isotope Laboratory, University of Waterloo, to look at stable isotope signatures of organic and inorganic sulfur.

Wild Fish:

We continued our yearly sampling of fish populations in Lake 375 and two reference lakes as in previous years. White sucker, slimy sculpin, and cyprinid populations were sampled in the spring and fall with trap nets. Lake trout were sampled in the fall with trap nets and short sets of small-mesh gillnets. Almost all fish were returned live to each lake. Each white sucker and lake trout was anaesthetized, weighed, measured, and marked before release. A few fin-rays were removed for age determinations from a representative sample of each species. Each cyprinid or slimy sculpin was anaesthetized and measured. Data are presently being analyzed.

As in the previous year, the barrier fence on the outflow of the study lake was inspected over the course of the field season to ensure that no escaped farm fish (experimental or accidental) could move downstream to Manomin Lake.

We continued to maintain three radio-linked acoustic fish positioning systems (VRAP, Vemco, Nova Scotia) that continually monitor the movements of acoustically-tagged fish in the study lake (375; two systems) and the reference lake (373; one system). We augmented the data collected using the automated positioning systems with the use of multiple passive receivers which record date and time, swimming depth, and unique fish identifier number.

We were once again faced with numerous mortalities of tagged fish, such that there is now one tagged lake trout and one tagged white sucker in Lake 375 and five tagged lake trout in Lake 373. In the fall of 2003, we released 10 rainbow trout into Lake 375. All rainbow trout died, most within one month after release. To examine whether or not the mortality rate of "escapees" was a result of the surgical transmitter implantation, we performed a "dummy" transmitter test in the fall of 2004. We fitted five rainbow trout with inactive transmitters, and kept these fish in a separate pen attached to the main cage. These "dummy" tagged fish were fed at the same rate as trout within the farm. At the same time, we released four rainbow trout with active transmitters into the lake. The fish in the "dummy" group lived for six weeks, at which point they were removed from L375 just prior to the annual harvest. The experimental escapees are still living in the study system. The longer survival in 2004 compared to 2003 could be attributable to a smaller tag size (which was only available in 2004), or to better growing conditions during the farming season, resulting in rainbow trout that were in much better condition in 2004 than in 2003.

We again examined cyprinid abundance and schooling behaviour through underwater visual observation and video recording. Fathead minnow spawning behaviour at the cage was also documented using video recording. Minnow trapping was conducted at the cage site, at littoral sites around Lake 375, and at similar sites in Lake 373. Length and weight data were recorded in order to examine size distribution of minnow species, and samples were preserved for stable isotope analysis.

Presentations and Publications:

There are no publications as yet from this project. Preliminary results have been presented at the following:

- CCFFR, 2003
- Ontario Aquaculture Association Annual General Meeting, 2003
- American Fisheries Society Annual Meeting, 2004
- Canadian Aquaculture Association Annual Meeting,2004
- Interprovincial Initiative for Sustainable Aquaculture Annual Meeting, 2004
- SETAC, 2004
- Ontario Federation of Anglers and Hunters, Executive Board Meeting

Plans for 2005:

Once our data sets are more complete, the project team will be better able to identify research priorities for the future. Currently, funding for this project ends in March 2005. A new proposal for additional years of funding will be submitted to ACRDP in January 2005; if that proposal is unsuccessful the project will not continue. Instead, limited monitoring of recovery will commence. A priority is to identify additional sources of research funds in order to ensure success of the project, as in the past ACRDP funding has been insufficient to complete all scientific components.

FOOD WEB INTERACTIONS

If we are to fully understand how human activities impact lake ecosystems and fish habitat, we must gain a better understanding of how the populations that make up lake food webs interact with each other. At the ELA, these questions are often addressed by graduate students as part of their theses requirements. During 2004, two such studies were underway.

IMPACT OF INVERTEBRATE PREDATORS ON CRUSTACEAN ZOOPLANKTON

Investigators:

- Dalila Seckar, M.Sc. candidate, Department of Entomology, University of Manitoba
- Dr. Michael Paterson, Research Scientist, Department of Fisheries and Oceans, Freshwater Institute

Study Goals:

Zooplankters have an important role in the food webs of freshwater lakes; they consume phytoplankton and are eaten in turn by invertebrate predators and fish. The goal of this project is to examine the interactions among three common invertebrate predators and to determine their impact on the zooplankton community using experimental mesocosms. Eighteen mesocosms were installed that contained different combinations of the invertebrate predators *Mysis relicta*, *Chaoborus* spp., and *Leptodora kindtii*.

Study Activities:

The goal of the summer 2004 activities was to install and monitor zooplankton and invertebrate predators in the study mesocosms. Enclosures of 1.58m in diameter and 11 to 12m deep were anchored to the bottom of lake 239. The enclosures were pumped full of water from the lake and stocked with zooplankton from L239. Three invertebrate predators were added to the eighteen enclosures in various combinations. *Mysis relicta* was collected from lake 239, *Chaoborus* spp. were collected from lake 227, and *Leptodora kindtii* was collected from lake 240 for these additions. Target densities in the enclosures were within the natural range of these predators in ELA lakes. Zooplankton were collected from the enclosures weekly and water for chemistry analyses was collected at the beginning, middle, and end of the experiment. Sampling lasted for six weeks and in the final sample week, collections for the invertebrate predators were conducted.

Future Plans:

Future plans will be assessed after more samples have been analysed. We are currently counting samples from the enclosures and awaiting the results of the chemical analyses. Possible future directions include a survey of predators and zooplankton communities in ELA lakes, or further mesocosm manipulations.

VIRAL ECOLOGY IN LAKES: VIRUSES THAT SPECIFICALLY INFECT PHYTOPLANKTON.

Principal Investigators:

- Jessica L. Clasen, Ph.D. Candidate, University of British Columbia, Dept. of Earth and Ocean Sciences.

- Dr. Curtis A. Suttle, thesis advisor, University of British Columbia, Departments of Earth and Ocean Sciences, Botany and Microbiology and Immunology.

Goals:

Viruses are abundant and dynamic members of aquatic ecosystems. Despite this knowledge, little is known about their ecology; this is particularly true in lake environments. My thesis research has two specific objectives designed to investigate the role viruses occupy in structuring phytoplankton community composition. I am particularly interested in viruses that infect phytoplankton (including cyanobacteria) because infections in these cells may dramatically affect ecosystem ecology because of the unique position phytoplankton occupy in aquatic environments. Therefore, understanding how viruses may structure phytoplankton community composition (for example how they may affect seasonal succession of phytoplankton) is important in order to infer the ecological significance of viruses.

The specific objectives of my thesis are:

1. To document the temporal variation in the viral community that infects phytoplankton using molecular techniques. This information will then be compared to basic limnological data (such as phytoplankton abundance and composition) to determine if changes in phytoplankton composition can be explained by variation in the virioplankton community.
2. To estimate the amount of phytoplankton mortality that can be attributed to viral infections. Several different experimental approaches will be used to estimate mortality rates, including modified zooplankton-grazing dilution experiments, viral production and enzymatic digestion assays.

Description of Research:

Objective 1. Temporal Variation

During the field season of 2004, I focused on four lakes at the ELA, including L224, L227, L239 and L240. Every week, I sampled these four lakes and determined viral, bacterial, Cyanobacterial and phytoplankton abundances. When the lakes were sampled for the LTER program (~every two weeks), I collected 20 L of water from each lake and made a viral concentrate (VC). A VC is made using a spiral wound tangential flow cartridge containing filters that have very small pore-sizes (in this case molecular weight cut-off of 10 kDa). Over the course of 10 hours, this cartridge slowly reduces the 20 L sample to about 200 mL, concentrating the viral community. I made a total of 42 VCs at the ELA during the field season of 2004. VCs will be further processed using molecular techniques to generate 'fingerprints' of the viral community. How these 'fingerprints' change over the season in each lake will then be compared to changes in the phytoplankton community to determine if viruses are important in structuring phytoplankton community composition.

Objective 2. Estimating Viral Mediated Phytoplankton Mortality.

I conducted mortality experiments on water collected from two ELA lakes (L227 and L239) during the 2004 field season. I conducted a total of 18 experiments using three different approaches, including viral dilution, viral production and enzymatic digestion experiments. Below is a brief description of each of the experimental approaches.

Viral Dilution Experiments:

The principal behind the viral dilution experiment is to create a gradient of both virus and host abundances by diluting whole lake water with ultra-clean (virus-free) lake water. The dilution process reduces the mortality pressure exerted by viruses allowing the phytoplankton to grow. The greater the dilution factor, the greater the reduction and, therefore, the more phytoplankton growth that should occur after incubation. Growth is measured in the gradient series and regressed against the dilution factor to determine mortality rates.

Viral Production Experiments:

In viral production experiments, the background viral abundance is reduced while maintaining ambient abundances of the other organisms (cyanobacteria and phytoplankton, in this case). Small changes in viral abundance are monitored over a short period of time (12 hours). The production of these new viruses is a result of cells infected at the onset of the experiments that die. The viral production rate (viruses/mL/hour) is determined from a first order regression analysis of viral abundance and time. The mortality rate of phytoplankton is then back calculated from the production rate to estimate phytoplankton mortality associated with viruses.

Enzymatic Digestion Experiment:

There is evidence that infected phytoplankton cells have comprised cell membranes. The enzymatic digestion method utilizes this information to determine the number of phytoplankton cells infected in a sample by digesting away infected cells with DNAse and Trypsin. By comparing the phytoplankton abundances and composition in enzymatic treated and untreated samples, viral mediated mortality is determined.

Figure V1. Abundance of viruses in the four sampled ELA lakes (L224, L227, L239 and L240). Samples were collected every week from mid-May to late October stained with SYBR Green and counted on an epifluorescent microscope.

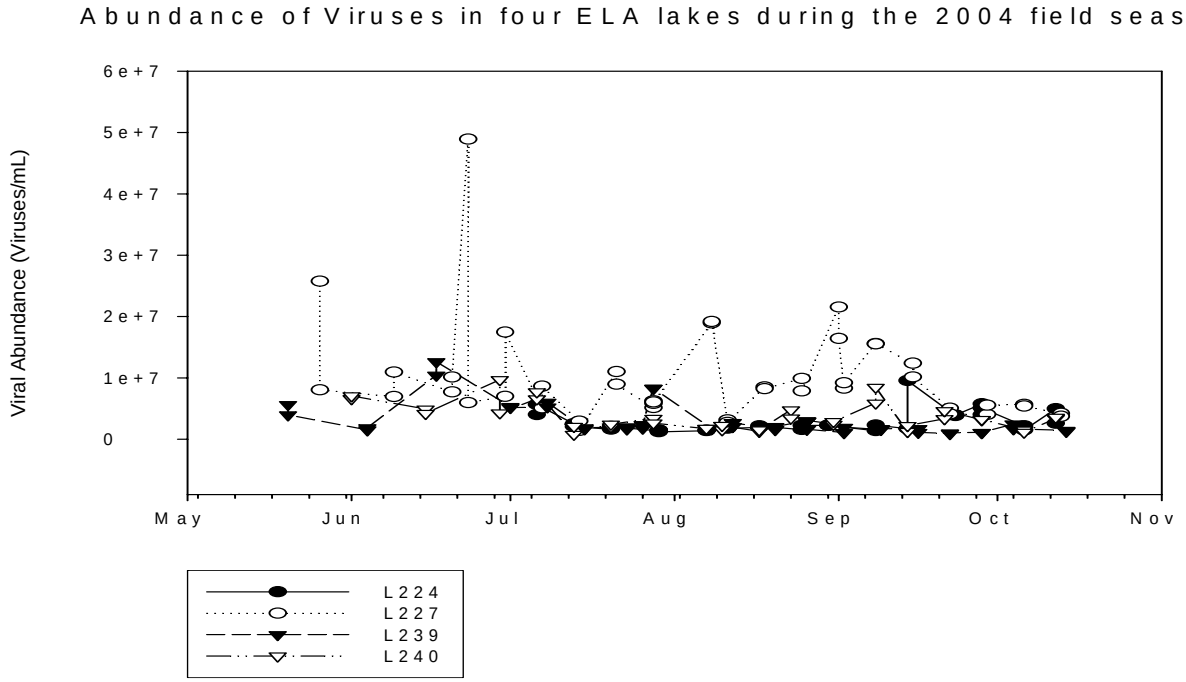

Figure V2. Abundance of bacteria in the four sampled ELA lakes (L224, L227, L239 and L240). Samples were collected every week from mid-May to late October stained with DAPI and counted on an epifluorescent microscope.

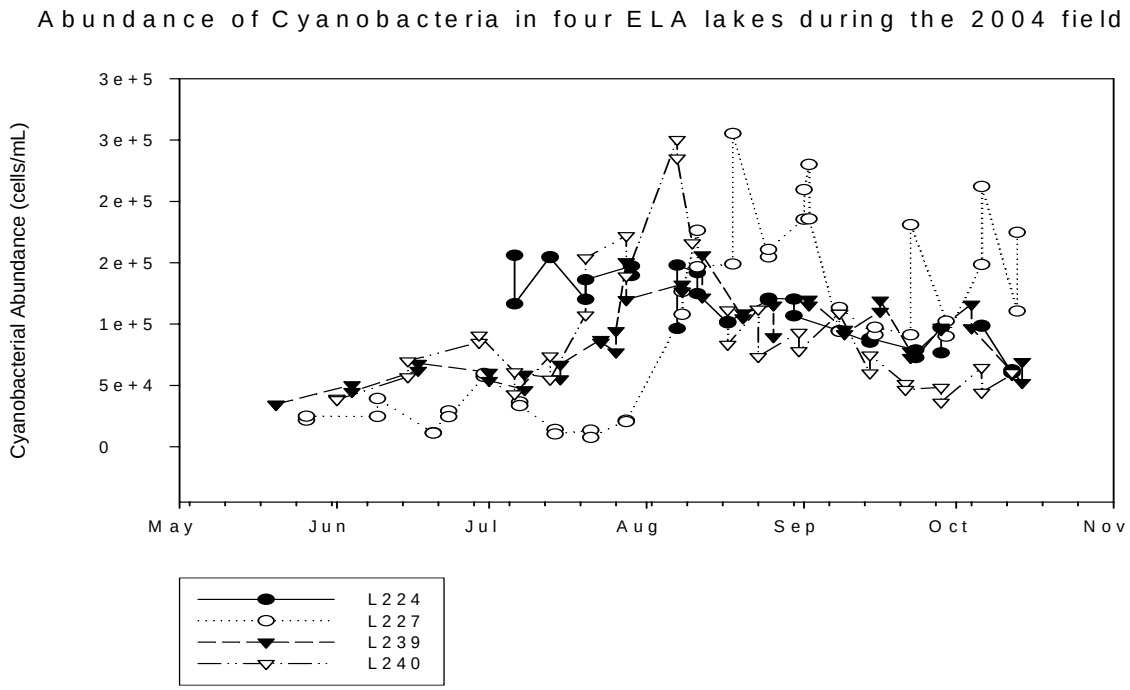

Figure V3. Abundance of unicellular cyanobacteria in the four sampled ELA lakes (L224, L227, L239 and L240). Samples were collected every week from mid-May to late October and counted on an epifluorescent microscope.